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File 155: MEDLINE(R) 1966-2002/Nov W3
*File 155: For updating information please see Help News155. Alert feature enhanced with customized scheduling. See HELP ALERT.
File 5:Biosis Previews(R) 1969-2002/Dec W2
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*File 5: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.

| Set | Items | Description |
|------------|-----------------------------------|-----------------------------------|
| ? s | py<1998 and ribozym? and liposom? | |
| Processing | | |
| 20949514 | PY<1998 | |
| 6250 | RIBOZYM? | |
| 52222 | LIPOSOM? | |
| S1 | 46 | PY<1998 AND RIBOZYM? AND LIPOSOM? |
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| | S2 | 32 RD (unique items) |
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| | 1534150 | NEOPLAS? |
| | 829518 | CANCER? |
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3/3,AB/1 (Item 1 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09645930 98069311 PMID: 9406237
Targeting gene therapy to **cancer**: a review.
Dachs G U; Dougherty G J; Stratford I J; Chaplin D J
Gray Laboratory, Mount Vernon Hospital, Northwood, UK.
dachs@graylab.ac.uk
Oncology research (UNITED STATES) 1997, 9 (6-7) p313-25,
ISSN 0965-0407 Journal Code: 9208097

Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

In recent years the idea of using gene therapy as a modality in the treatment of diseases other than genetically inherited, monogenic disorders has taken root. This is particularly obvious in the field of oncology where currently more than 100 clinical trials have been approved worldwide. This report will summarize some of the exciting progress that has recently been made with respect to both targeting the delivery of potentially therapeutic genes to tumor sites and regulating their expression within the tumor microenvironment. In order to specifically target malignant cells while at the same time sparing normal tissue, **cancer** gene therapy will need to combine highly selective gene delivery with highly specific gene expression, specific gene product activity, and, possibly, specific drug activation. Although the efficient delivery of DNA to tumor sites remains a formidable task, progress has been made in recent years using both viral (retrovirus, adenovirus, adeno-associated virus) and nonviral (liposomes, gene gun, injection) methods. In this report emphasis will

be placed on targeted rather than high-efficiency delivery, although those would need to be combined in the future for effective therapy. To date delivery has been targeted to tumor-specific and tissue-specific antigens, such as epithelial growth factor receptor, c-kit receptor, and folate receptor, and these will be described in some detail. To increase specificity and safety of gene therapy further, the expression of the therapeutic gene needs to be tightly controlled within the target tissue. Targeted gene expression has been analyzed using tissue-specific promoters (breast-, prostate-, and melanoma-specific promoters) and disease-specific promoters (carcinoembryonic antigen, HER-2/neu, Myc-Max response elements, DF3/MUC). Alternatively, expression could be regulated externally with the use of radiation-induced promoters or tetracycline-responsive elements. Another novel possibility that will be discussed is the regulation of therapeutic gene products by tumor-specific gene splicing. Gene expression could also be targeted at conditions specific to the tumor microenvironment, such as glucose deprivation and hypoxia. We have concentrated on hypoxia-targeted gene expression and this report will discuss our progress in detail. Chronic hypoxia occurs in tissue that is more than 100-200 microns away from a functional blood supply. In solid tumors hypoxia is widespread both because cancer cells are more prolific than the invading endothelial cells that make up the blood vessels and because the newly formed blood supply is disorganized. Measurements of oxygen partial pressure in patients' tumors showed a high percentage of severe hypoxia readings (less than 2.5 mmHg), readings not seen in normal tissue. This is a major problem in the treatment of cancer, because hypoxic cells are resistant to radiotherapy and often to chemotherapy. However, severe hypoxia is also a physiological condition specific to tumors, which makes it a potentially exploitable target. We have utilized hypoxia response elements (HRE) derived from the oxygen-regulated phosphoglycerate kinase gene to control gene expression in human tumor cells in vitro and in experimental tumors. The list of genes that have been considered for use in the treatment of cancer is extensive. It includes cytokines and costimulatory cell surface molecules intended to induce an effective systemic immune response against tumor antigens that would not otherwise develop. Other inventive strategies include the use of internally expressed antibodies to target oncogenic proteins (intrabodies) and the use of antisense technology (antisense oligonucleotides, antigenes, and ribozymes). This report will concentrate more on novel genes encoding prodrug activating enzymes, so-called suicide genes (Herpes simplex virus thymidine kinase, Escherichia coli nitroreductase, E. (ABSTRACT TRUNCATED)

3/3, AB/2 (Item 2 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08897468 96263414 PMID: 8653480

Antisense strategies and therapeutic applications.

Putnam D A

Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA.

American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists (UNITED STATES) Jan 15 1996, 53 (2) p151-60; quiz 182-3, ISSN 1079-2082 Journal Code: 9503023

Contract/Grant No.: GM08393; GM; NIGMS

Erratum in Am J Health Syst Pharm 1996 Feb 1;53(3) 325

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The concepts underlying the antisense approach to disease therapy are discussed, and potential applications are examined. Antisense therapeutic agents bind to DNA or RNA sequences, blocking the synthesis of cellular

proteins with unparalleled specificity. Transcription and translation are the two processes with which the agents interfere. There are three major classes of antisense agents: antisense sequences, commonly called antisense oligonucleotides; antigenic sequences; and **ribozymes**. Antisense sequences are derivatives of nucleic acids that hybridize cytosolic messenger RNA (mRNA) sense strands through hydrogen bonding to complementary nucleic acid bases. Antigenic sequences hybridize double-stranded DNA in the nucleus, forming triple helices. **Ribozymes**, rather than inhibiting protein synthesis simply by binding to a single targeted mRNA, combine enzymatic processes with the specificity of antisense base pairing, creating a molecule that can incapacitate multiple targeted mRNAs. Antisense therapeutic agents are being investigated in vitro and in vivo for use in treating human immunodeficiency virus infection, hepatitis B virus infection, herpes simplex virus infection, papillomavirus infection, **cancer**, restenosis, rheumatoid arthritis, and allergic disorders. Although many results are preliminary, some are promising and have led to clinical trials. A major goal in developing methods of delivering antisense agents is to reduce their susceptibility to nucleases while retaining their ability to bind to targeted sites. Modification of the phosphodiester linkages in oligonucleotides can lend the sequences enzymatic stability without affecting their binding capacities. Carrier systems designed to protect the antisense structure and improve passage through the cell membrane include **liposomes**, water-soluble polymers, and nanoparticles. The pharmacokinetics of antisense agents are under investigation. Antisense therapeutic agents have the potential to become an integral part of medicinal regimens.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08581525 95346988 PMID: 7621511

Inhibition of gene expression with **ribozymes**.

Marschall P; Thomson J B; Eckstein F

Max-Planck-Institut fur Experimentelle Medizin, Gottingen, Germany.

Cellular and molecular neurobiology (UNITED STATES) Oct 1994, 14

(5) p523-38, ISSN 0272-4340 Journal Code: 8200709

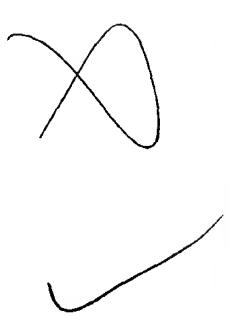
Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

1. **Ribozymes** can be designed to cleave in trans, i.e. several substrate molecules can be turned over by one molecule of the catalytic RNA. Only small molecular weight **ribozymes**, or small **ribozymes**, are discussed in this review with particular emphasis on the hammerhead **ribozyme** as this has been most widely used for the inhibition of gene expression by cleavage of mRNAs. 2. Cellular delivery of the **ribozyme** is of crucial importance for the success of inhibition of gene expression by this methodology. Two modes of delivery can be envisaged, endogenous and exogenous delivery. Of the former several variants exist, depending on the vector used. The latter is still in its infancy, even though chemical modification has rendered such **ribozymes** resistant against degradation by serum nucleases without impairment of catalytic efficiency. 3. Various successful applications of **ribozymes** for the inhibition of gene expression are discussed, with particular emphasis on HIV1 and **cancer** targets. These examples demonstrate the promise of this methodology.



3/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08478976 95235041 PMID: 7718886

Multi-unit **ribozyme**-mediated cleavage of bcr-abl mRNA in myeloid

leukemias.

Leopold L H; Shore S K; Newkirk T A; Reddy R M; Reddy E P
Temple University Hospital, Philadelphia, PA, USA.
Blood (UNITED STATES) Apr 15 1995, 85 (8) p2162-70, ISSN
0006-4971 Journal Code: 7603509

Contract/Grant No.: 1 RO1 5P01CA21124-15; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Chronic myelogenous leukemia is characterized by the Philadelphia chromosome, which at the molecular level results from the fusion of the bcr gene on chromosome 22 and the abl gene on chromosome 9. The bcr-abl fusion gene encodes a novel tyrosine kinase with transforming activity. In this study, we have synthesized a multi-unit ribozyme that targets bcr-abl mRNA. In vitro ribozyme cleavage reactions show increased cleavage efficiency of this multi-unit ribozyme compared with single or double ribozymes. The multiunit ribozyme was then transfected into murine myeloblasts transformed with the bcr-abl gene (32D cells). Ribozyme transfection was accomplished either by liposomes or using folate acid-polylysine as a carrier. Multi-unit ribozyme transfection reduced the level of bcr-abl mRNA 3 logs when transfected via folate receptor-mediated uptake into transformed 32D cells. These results suggest that a multi-unit ribozyme could be an effective therapeutic agent for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08253033 95009966 PMID: 7925305

Ribozyme -mediated cleavage of the MDR-1 transcript restores chemosensitivity in previously resistant cancer cells.

Kiehntopf M; Brach M A; Licht T; Petschauer S; Karawajew L; Kirschning C; Herrmann F

Department of Medical Oncology and Applied Molecular Biology, Freie Universität Berlin, Universitätsklinikum Rudolf Virchow, Robert-Rosse-Cancer Center, Germany.

EMBO journal (ENGLAND) Oct 3 1994, 13 (19) p4645-52, ISSN
0261-4189 Journal Code: 8208664

Retraction in EMBO J. 1997 Jul 1;16(13) 4153; Retraction in PMID 9280722

Document type: Journal Article; Retracted Publication

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

How cancer cells become resistant to chemotherapy is not completely understood, but it is believed that resistance is usually associated with overexpression of drug resistance genes. Drug resistance mediated by the MDR-1 gene is the first well characterized form of drug resistance in human cancer. MDR-1 encodes a phosphoglycoprotein, P-GP, that serves as an energy-dependent drug efflux pump, reducing intracellular drug accumulation and thereby cytotoxicity. We have used ribozymes to reverse the multiple drug resistance phenotype. A hammerhead ribozyme recognizing the GUC sequence at position -6 to -4 close to the translation start site of the 4.5 kb MDR-1 mRNA was prepared by in vitro transcription (MDR-1-RZiv) or chemical synthesis (MDR-1-RZs). Both MDR-1-RZiv and MDR-1-RZs specifically cleaved the MDR-1 mRNA into two parts of the expected size under physiological conditions in an extracellular system with MDR-1-RZiv being more effective. Site-specific cleavage was dependent on time, temperature and [MgCl₂]. To examine the in vivo potential of MDR-1-RZ, MDR-1-RZiv and MDR-1-RZs were transfected into a human pleural mesothelioma cell line and into one adriamycin-resistant and one vindesine-resistant subline thereof by liposome-mediated transfer.

09/10/98 1964 ✓

Incorporation of ribozymes resulted in significantly reduced expression of the MDR-1 gene, with MDR-1-RZs being more potent than MDR-1-RZiv in vitro. MDR-1-RZ reduces P-GP overexpression at the protein level. Liposome-mediated transfer of MDR-1-RZiv or MDR-1-RZs reversed the multiple drug resistance phenotype and restored sensitivity towards chemotherapeutic drugs.

3/3,AB/6 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

11098685 BIOSIS NO.: 199799719830
Receptor ligand-facilitated cationic liposome delivery of anti-HIV-1 Rev-binding aptamer and ribozyme DNAs.
AUTHOR: Konopka K(a); Duzgunes N; Rossi I J; Lee N S
AUTHOR ADDRESS: (a)Univ. Pacific, San Francisco, CA**USA
JOURNAL: FASEB Journal 11 (9):pA1090 1997
CONFERENCE/MEETING: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English
1997

3/3,AB/7 (Item 2 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

10723692 BIOSIS NO.: 199799344837
Methods in Molecular Medicine: Gene therapy protocols.
BOOK TITLE: Methods in Molecular Medicine; Gene therapy protocols
AUTHOR: Robbins Paul D
BOOK AUTHOR/EDITOR: Robbins P D: Ed
AUTHOR ADDRESS: Dep. Mol. Genet. Biochem., Univ. Pittsburgh, Pittsburgh, PA**USA
pxiv+432p 1997
BOOK PUBLISHER: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA
ISBN: 0-89603-307-4 (paper); 0-89603-484-4 (cloth)
DOCUMENT TYPE: Book; Manual
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This book is part of a series of advanced instruction manuals on diverse topics in molecular medicine. The thirty chapters contained in this volume house practices and protocols useful for investigations involving gene therapy. The chapters contain an introduction, lists of materials, step-wise instructions, notes housing explanations and helpful hints, and references. Topics include: the use of DNA adenovirus conjugates, replication-defective Herpes vectors, liposome-mediated gene transfer, cytokine gene-modified tumor cells in cancer therapy, and suppression of carcinoma by an antioncogene ribozyme. The text is illustrated and indexed.

1997

3/3,AB/8 (Item 3 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

10357725 BIOSIS NO.: 199698812643

Reversal of multidrug resistance by a **liposome-MDR1 ribozyme** complex.

AUTHOR: Masuda Y(a); Kobayashi H; Holland J F(a); Ohnuma T

AUTHOR ADDRESS: (a)Mount Sinai Med. Center, New York, NY 10029**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 37 (0):p353-354 1996

CONFERENCE/MEETING: 87th Annual Meeting of the American Association for Cancer Research Washington, D.C., USA April 20-24, 1996

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

1996

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Set Items Description
S1 46 PY<1998 AND RIBOZYM? AND LIPOSOM?
S2 32 RD (unique items)
S3 8 S2 AND (NEOPLAS? OR CANCER?)

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32 S2

8 S3

S4 24 S2 NOT S3

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4/3,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09634308 98044835 PMID: 9383578

Exogenous application of **ribozymes** for inhibiting gene expression.

Eckstein F

Max-Planck Institute of Experimental Medicine, Gottingen, Germany.
Ciba Foundation symposium (NETHERLANDS) 1997, 209 p207-12;
discussion 212-7, ISSN 0300-5208 Journal Code: 0356636

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Sequence-specific inhibition of gene expression is an attractive concept for the development of a new generation of therapeutics. Two alternatives can be envisaged for the introduction of **ribozymes** into cells: endogenous or exogenous delivery. In the latter, the **ribozyme** is prepared by chemical synthesis or transcription and delivered to the cell either unaided or with the help of **liposomes**. A problem with this approach is the abundance of RNases in the serum, and thus the stabilization of the **ribozyme** is necessary but without the impairment of catalytic efficiency. This has been achieved by several groups by 2'-modification of the pyrimidine nucleosides and the introduction of a few phosphorothioates at the termini. The selection of **ribozyme**-accessible sites on the target and the attachment of cholesterol and peptides to the **ribozymes** will be discussed. Examples of the application of these modified **ribozymes** in cell cultures will be presented, including the inhibition of expression of the multiple drug resistance gene, after unaided as well as **liposome**-aided delivery, and studies of animal models demonstrating the potential of this particular application strategy.

4/3,AB/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09607244 98025254 PMID: 9360779

Ribozyme-mediated inhibition of a Philadelphia chromosome-positive

acute lymphoblastic leukemia cell line expressing the p190 bcr-abl oncogene.

Snyder D S; Wu Y; McMahon R; Yu L; Rossi J J; Forman S J

Department of Hematology and Bone Marrow Transplantation, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, USA.

Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation (UNITED STATES) Oct 1997, 3 (4) p179-86, ISSN 1083-8791 Journal Code: 9600628

Contract/Grant No.: CA30206; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The bcr-abl oncogene is the molecular counterpart of the Philadelphia chromosome (Ph), which is detected in > 95% of patients with chronic myelogenous leukemia (CML) and 20-30% of adults with acute lymphoblastic leukemia (ALL). Leukemic cells from patients with CML express the p210 form of the bcr-abl oncogene, whereas in adult Ph+ ALL approximately 50% of cases express the p190 form of the bcr-abl oncogene, and the other 50% express the same p210 gene as is found in CML. In this study, we have designed hairpin **ribozymes** (RZs) specific for the p190 form of the bcr-abl oncogene to inhibit the growth of a p190 Ph+ ALL cell line, Sup-B15. The RZs cleave p190 RNA substrate in a cell-free in vitro assay. In the presence of the **liposome**, DMRIE-C, the RZs are protected from serum mediated catalysis in vitro. Anti-p190 RZs transfected with DMRIE-C as the vector into K562 cells, which express the p210 bcr-abl oncogene, are stable intracellularly for up to 96 hours. Up to 33% of the DMRIE-C and RZ mixtures are taken up by Sup-B15 cells cultured in suspension. Expression of the p190 bcr-abl protein product is specifically inhibited as demonstrated by Western blot analysis. Cell growth of the Sup-B15 cells is completely inhibited by anti-p190 RZs over four days in culture. Anti-p210 RZs have no significant effect on bcr-abl protein expression or cell growth by Sup-B15 cells. RZs may have a role in purging stem cell populations collected from patients with Ph+ ALL in the context of autologous bone marrow transplantation.

4/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09567057 98001685 PMID: 9341150

Efficient transfer of synthetic **ribozymes** into cells using hemagglutinating virus of Japan (HVJ)-cationic **liposomes**. Application for **ribozymes** that target human t-cell leukemia virus type I tax/rex mRNA.

Kitajima I; Hanyu N; Soejima Y; Hirano R; Arahira S; Yamaoka S; Yamada R; Maruyama I; Kaneda Y

Department of Laboratory and Molecular Medicine, University of Kagoshima, 8-35-1 Sakuragaoka, Kagoshima 890, Japan.

Journal of biological chemistry (UNITED STATES) Oct 24 1997, 272 (43) p27099-106, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We investigated the usefulness of **ribozymes** in inhibiting the expression of human T-cell leukemia virus type I (HTLV-I) gene. Two hammerhead **ribozymes** that were against HTLV-I rex (RR) and tax (TR) mRNA were synthesized. Both **ribozymes** were sequence-specific in the in vitro cleavage analysis of run-off transcripts from tax/rex cDNA. Intracellular activities of the **ribozymes** were studied in HTLV-I tax cDNA-transfected rat embryonic fibroblasts (Rat/Tax cells), which expressed the Tax but not Rex. **Ribozymes** were delivered into cells using anionic or cationic **liposomes** fused with hemagglutinating virus of

Japan (HVJ). Cellular uptake of **ribozymes** complexed with HVJ-cationic **liposomes** was 15-20 times higher cellular uptake than naked **ribozymes**, and 4-5 times higher than that of **ribozymes** complexed with HVJ-anionic **liposomes**. HVJ-cationic **liposomes** promoted accumulation of **ribozymes** in cytoplasm and accelerated transport to the nucleus. Tax protein levels were decreased about 95% and were five times lower when the same amount of TR was introduced into the cells using HVJ-cationic, rather than HVJ-anionic **liposomes**. Inactive **ribozyme** and tax antisense oligodeoxynucleotides reduced Tax expression by about 20%, whereas RR and tax sense oligodeoxynucleotides had no effect. These results suggest that the **ribozymes'** effect against tax mRNA was sequence-specific, and HVJ-cationic **liposomes** can be useful for intracellular introduction of **ribozymes**.

4/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09516882 97422463 PMID: 9278231

Use of a hammerhead **ribozyme** with cationic **liposomes** to reduce leukocyte type 12-lipoxygenase expression in vascular smooth muscle.

Gu J L; Nadler J; Rossi J
Department of Diabetes, Endocrinology and Metabolism, City of Hope Medical Center, Duarte, CA 91010, USA.

Molecular and cellular biochemistry (NETHERLANDS) Jul 1997, 172 (1-2) p47-57, ISSN 0300-8177 Journal Code: 0364456

Contract/Grant No.: AI29329; AI; NIAID; P01-HL 55798; HL; NHLBI; R01-DK-39721; DK; NIDDK; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Chemically synthesized hammerhead-type **ribozymes** targeted against the porcine leukocyte-type 12-lipoxygenase (LO) have been developed and studied. One chimeric **ribozyme** consists of DNA in the non-enzymatic portions, and RNA in the enzymatic core as well as two phosphorothioate internucleotide linkages at 3' terminus. The second **ribozyme** consists of ribonucleotide sequences generated by in vitro transcription. In this chapter we describe methodologies to first analyze the **ribozyme** catalytic activity in vitro by studying cleavage of target RNA in vitro. The subsequent sections will describe how to target the catalytic **ribozyme** and deliver it to porcine vascular smooth muscle cells (PVSMC) by a **liposome**-mediated method. Finally ways to evaluate its activity to inhibit expression of the 12-LO mRNA will be presented. These results demonstrate the feasibility of using **ribozymes** as novel candidates for therapeutic agents to block specific gene expression in vascular cells.

4/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09457781 97348448 PMID: 9204458

Exogenous cellular delivery of **ribozymes** and **ribozyme** encoding DNAs.

Castanotto D; Bertrand E; Rossi J
Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA, USA.

Methods in molecular biology (Clifton, N.J.) (UNITED STATES) 1997, 74 p429-39, ISSN 1064-3745 Journal Code: 9214969

Contract/Grant No.: AI 25959; AI; NIAID; AI 29329; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

4/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09270491 97153015 PMID: 8999949

Sterically stabilized pH-sensitive **liposomes**. Intracellular delivery of aqueous contents and prolonged circulation *in vivo*.

Slepushkin V A; Simoes S; Dazin P; Newman M S; Guo L S; Pedrosa de Lima M C; Duzgunes N

Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, California 94115, USA.

Journal of biological chemistry (UNITED STATES) Jan 24 1997, 272

(4) p2382-8, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: AI 32399; AI; NIAID; AI 33833; AI; NIAID; AI 35231; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Liposomes that destabilize at mildly acidic pH are efficient tools for delivering water-soluble drugs into the cell cytoplasm. However, their use *in vivo* is limited because of their rapid uptake from circulation by the reticuloendothelial system. Lipid-anchored polyethylene glycol (PEG-PE) prolongs the circulation time of **liposomes** by steric stabilization.

We have found that addition of PEG-PE to the membrane of pH-sensitive **liposomes** composed of cholesteryl hemisuccinate (CHEMS) and dioleylphosphatidylethanolamine (DOPE) confers steric stability to these vesicles. This modification significantly decreases the pH-dependent release of a charged water-soluble fluorophore, calcein, from **liposomes** suspended in buffer or cell culture medium. However, the ability of such **liposomes** to release calcein intracellularly, measured by a novel flow cytometry technique involving dual fluorescence labeling, remains unaltered. As expected, the release of calcein from **liposomes** endocytosed by cells is inhibited upon pretreatment of the cells with NH₄Cl, an inhibitor of endosome acidification. The unique properties of these **liposomes** were also demonstrated *in vivo*. The distribution kinetics of ¹¹¹In-containing CHEMS/DOPE/PEG-PE liposomes injected intravenously into rats has pharmacokinetic parameters similar to control, non-pH-sensitive, sterically stabilized CHEMS/distearoylphosphatidylcholine/PEG-PE **liposomes**. In contrast, regular pH-sensitive **liposomes** lacking the PEG-PE component are cleared rapidly. Sterically stabilized pH-sensitive liposomes may therefore be useful for the intracellular delivery *in vivo* of highly negatively charged molecules such as genes, antisense oligonucleotides, and ribozymes for the treatment of various diseases.

4/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

09258736 97171202 PMID: 9018457

Progress, problems, and prospects for gene therapy in muscle.

Brown R H; Miller J B

Neuromuscular Laboratory, Massachusetts General Hospital, Charlestown 02129, USA.

Current opinion in rheumatology (UNITED STATES) Nov 1996, 8 (6)
p539-43, ISSN 1040-8711 Journal Code: 9000851

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

As the molecular defects that cause many muscle diseases have been

identified, research has shifted to finding novel therapies. Gene therapy has been proposed both for correcting primary gene defects of muscle and as a way of using muscle for the production of proteins therapeutic in inflammatory or nonmuscle diseases. Several strategies have been developed to introduce foreign genes into diseased muscles, including myoblast transfer, direct injection of plasmids or DNA-liposome complexes, and infection with modified viruses. Related strategies, using antisense sequences or **ribozymes**, have been devised to modify gene expression in diseased cells. No method has yet proved itself in the clinic, although current work remains promising and some of the pitfalls that must be overcome have been identified.

4/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08959925 96313822 PMID: 8703987

Human immunodeficiency virus type-1 (HIV-1) infection increases the sensitivity of macrophages and THP-1 cells to cytotoxicity by cationic liposomes.

Konopka K; Pretzer E; Felgner P L; Duzgunes N
Department of Microbiology, University of the Pacific School of Dentistry, San Francisco, CA 94115, USA.

Biochimica et biophysica acta (NETHERLANDS) Jul 24 1996, 1312

(3) p186-96, ISSN 0006-3002 Journal Code: 0217513

Contract/Grant No.: RO1 AI-32399; AI; NIAID; UO1 AI-35231; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cationic liposomes may be valuable for the delivery of anti-sense oligonucleotides, **ribozymes**, and therapeutic genes into human immunodeficiency virus type 1 (HIV-1)-infected and uninfected cells. We evaluated the toxicity of three cationic liposomal preparations, Lipofectamine, Lipofectin, and 1, 2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE) reagent, to HIV-infected and uninfected cells. Monocyte/macrophages were infected with HIV-1BaL and treated with liposomes in medium containing 20% fetal bovine serum (FBS) for 4 h or 24 h at 37 degree C. Uninfected monocytic THP-1 cells and chronically infected THP-1/HIV-1IIIB cells were treated with phorbol 12-myristate 13-acetate (PMA) and exposed to liposomes in the presence of 10% FBS. Toxicity was evaluated by the Alamar Blue assay and viral p24 production. The toxic effect of cationic liposomes was very limited with uninfected cells, although concentrations of liposomes that were not toxic within a few days of treatment could cause toxicity at later times. In HIV-1BaL-infected macrophages, Lipofectamine (up to 8 microM) and Lipofectin (up to 40 microM) were not toxic after a 4-h treatment, while DMRIE reagent at 40 microM was toxic. While a 4-h treatment of THP-1/HIV-1IIIB cells with the cationic liposomes was not toxic, even up to 14 days post-treatment, all three cationic liposomes were toxic to cells at the highest concentration tested after a 24-h treatment. Similar results were obtained with the Alamar Blue assay, Trypan Blue exclusion and a method that enumerates nuclei. Infected cells with relatively high overall viability could be impaired in their ability to produce virions, indicating that virus production appears to be more sensitive to treatment with the cationic liposomes than cell viability. Our results indicate that HIV-infected cells are more susceptible than uninfected cells to killing by cationic liposomes. The molecular basis of this differential effect is unknown; it is proposed that alterations in cellular membranes during virus budding cause enhanced interactions between cationic liposomes and cellular membranes.

4/3,AB/9 (Item 9 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

08877317 96238059 PMID: 8647163

Ribozyme modulation of lipopolysaccharide-induced tumor necrosis factor-alpha production by peritoneal cells in vitro and in vivo.

Sioud M
Institute of Immunology and Rheumatology, National Hospital, Oslo,
Norway.

European journal of immunology (GERMANY) May 1996, 26 (5)
p1026-31, ISSN 0014-2980 Journal Code: 1273201

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have utilized synthetic ribozymes to modulate the lipopolysaccharide (LPS)-induced production of tumor necrosis factor-alpha (TNF-alpha) by peritoneal cells. Two hammerhead ribozymes (mRz1 and mRz2) were prepared by transcription in vitro and their activities in vitro and in vivo were investigated. Both ribozymes cleaved their RNA target with an apparent turnover number (kcat) of 2 min(-1), and inhibited TNF-alpha gene expression in vitro by 50% and 70%, respectively. When mRz1 and mRz2, entrapped in liposomes, were delivered into mice by intraperitoneal injection, they inhibited LPS-induced TNF-alpha gene expression in vivo with mRz2 being the most effective. This enhanced activity could result from the facilitation of catalysis by cellular endogenous proteins, since they specifically bind to mRz2 as compared to mRz1. Furthermore, a significant mRz2 activity can be recovered from peritoneal cells 2 days post-administration in vivo. The anti-TNF-alpha ribozyme treatment in vivo resulted in a more significant reduction of LPS-induced IFN-gamma protein secretion compared to IL-10. In contrast to this pleiotropic effect, the anti-TNF-alpha ribozyme treatment did not affect the heterogenous expression of Fas ligand by peritoneal cells, indicating the specificity of the treatment. Taken together, the present data indicate that the biological effects of TNF-alpha can be modulated by ribozymes. In addition, the data suggest that ribozymes can be administered in a drug-like manner, and therefore indicate their potential in clinical applications.

4/3, AB/10 (Item 10 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

08548625 95308703 PMID: 7540514

Ribozyme-mediated inhibition of expression of leukocyte-type 12-lipoxygenase in porcine aortic vascular smooth muscle cells.

Gu J L; Veerapanane D; Rossi J; Natarajan R; Thomas L; Nadler J
Department of Diabetes, Endocrinology, and Metabolism, City of Hope Medical Center, Duarte, CA 91010, USA.

Circulation research (UNITED STATES) Jul 1995, 77 (1) p14-20,

ISSN 0009-7330 Journal Code: 0047103

Contract/Grant No.: R01-DK-39721; DK; NIDDK; R29-HL-48920; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Activation of a leukocyte-type 12-lipoxygenase (12-LO) has been proposed to be an important mechanism for angiotensin II- and glucose-induced vascular smooth muscle cell growth. Currently, no specific pharmacological inhibitors for the leukocyte-type 12-LO are available to test this hypothesis. We have therefore designed a chimeric DNA-RNA hammerhead ribozyme to produce cleavage at the first GUC sequence at nucleotide 7 of porcine leukocyte 12-LO mRNA. The ribozyme was tested in vitro with a 206-base 12-LO mRNA as substrate. We observed that the ribozyme specifically and dose-dependently cleaved porcine leukocyte

12-LO mRNA at the predicted site under physiological temperature. Furthermore, we also efficiently delivered the **ribozyme** into porcine aortic vascular smooth muscle cells by transfection with cationic **liposomes**. The **ribozyme** caused a dose-dependent decrease in levels of porcine leukocyte-type 12-LO mRNA in these cells and was more potent than an antisense oligonucleotide directed against porcine leukocyte 12-LO. The 12-LO **ribozyme** also attenuated 12-LO protein levels in the cells. The action of the **ribozyme** was primarily a result of its catalytic activity, since a modified **ribozyme** that lacks catalytic activity showed reduced effects. This represents the first **ribozyme** directed against a mammalian LO pathway. These results demonstrate the potential utility of new **ribozyme** technology to generate novel agents for gene modulation experiments to modify the development or progression of vascular disease in humans.

4/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

08241693 95001928 PMID: 7918434

Functional characterization of **ribozymes** expressed using U1 and T7 vectors for the intracellular cleavage of ANF mRNA.

De Young M B; Kincade-Denker J; Boehm C A; Riek R P; Mamone J A; McSwiggen J A; Graham R M

Department of Cardiovascular Biology, Cleveland Clinic Research Institute, Ohio 44195.

Biochemistry (UNITED STATES) Oct 11 1994, 33 (40) p12127-38,
ISSN 0006-2960 Journal Code: 0370623

Contract/Grant No.: HL33713; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hammerhead **ribozymes** targeted to various GUC or GUA sites on rat atrial natriuretic factor (ANF) mRNA were developed. The catalytic activity of **ribozymes** to four of these sites, synthesized by transcription off synthetic oligodeoxynucleotide duplexes, was studied in detail. In vitro, **ribozyme**-mediated cleavage was highly Mg(2+)-dependent, and at concentrations approaching those found intracellularly, the rate but not the extent of cleavage was markedly reduced. To test for cellular activity, synthetic genes encoding the **ribozymes** were cloned between the initiation and termination sequences of the U1snRNA gene or between the T7RNA polymerase promoter and terminator sequences in pSP64. Both constructs had defined initiation and termination sequences to minimize transcript size and for message stability. In vitro the addition of T7 or U1 terminator sequences had variable effects on catalytic activity, presumably due to structural interactions between the **ribozyme** and the added sequence. The **ribozyme**-encoding plasmids were cotransfected with an expression plasmid containing a rat ANF cDNA into COS-1 cells using a **liposome** method, which provided high-level transfection efficiency. Quantitation of ANF mRNA by RNase protection showed marked decreases in ANF transcript levels with both the U1- and the T7-expressed **ribozymes** directed at three of the four sites on ANF mRNA. With all constructs, target accessibility, determined in vitro, was a more important determinant of intracellular ANF mRNA cleavage than catalytic activity per se. ANF mRNA cleavage was not merely due to an antisense effect, since a mutant construct that was catalytically inactive but could still bind produced less cleavage than the corresponding wild-type **ribozyme** construct. These findings indicate that both U1 and T7 vector systems provide efficient **ribozyme** expression for the intracellular cleavage of target mRNA.

4/3,AB/12 (Item 12 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

07793699 93320442 PMID: 8329715

Ribozyme-mediated inhibition of bcr-abl gene expression in a Philadelphia chromosome-positive cell line.

Snyder D S; Wu Y; Wang J L; Rossi J J; Swiderski P; Kaplan B E; Forman S J

Department of Hematology and Bone Marrow Transplantation, City of Hope National Medical Center, Duarte, CA 91010-0269.

Blood (UNITED STATES) Jul 15 1993, 82 (2) p600-5, ISSN

0006-4971 Journal Code: 7603509

Contract/Grant No.: CA 33572; CA; NCI; CA 59308; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The bcr-abl fusion gene is the molecular counterpart of the Philadelphia chromosome (Ph1) and is directly involved in the pathogenesis of Ph1+ leukemia. Inhibition of bcr-abl gene expression may have profound effects on the cell biology of Ph1+ cells, as recent experiments with antisense oligonucleotides have shown. In this study we have designed and synthesized a unique ribozyme that is directed against bcr-abl mRNA. The ribozyme cleaved bcr-abl mRNA in a cell-free in vitro system. A DNA-RNA hybrid ribozyme was then incorporated into a liposome vector and transfected into EM-2 cells, a cell line derived from a patient with blast crisis of chronic myelogenous leukemia. The ribozyme decreased levels of detectable bcr-abl mRNA in these cells, inhibited expression of the bcr-abl gene product, p210bcr-abl, and inhibited cell growth. This anti-bcr-abl ribozyme may be a useful tool to study the cell biology of Ph1+ leukemia and may ultimately have therapeutic potential in treating patients with Ph1 leukemias.

4/3,AB/13 (Item 13 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

07503086 93027153 PMID: 1408757

Chimeric DNA-RNA hammerhead ribozymes have enhanced in vitro catalytic efficiency and increased stability in vivo.

Taylor N R; Kaplan B E; Swiderski P; Li H; Rossi J J

Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Nucleic acids research (ENGLAND) Sep 11 1992, 20 (17) p4559-65
ISSN 0305-1048 Journal Code: 0411011

Contract/Grant No.: AI25959; AI; NIAID; AI29329; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subsequent to the discovery that RNA can have site specific cleavage activity, there has been a great deal of interest in the design and testing of trans-acting catalytic RNAs as both surrogate genetic tools and as therapeutic agents. We have been developing catalytic RNAs or ribozymes with target specificity for HIV-1 RNA and have been exploring chemical synthesis as one method for their production. To this end, we have chemically synthesized and experimentally analyzed chimeric catalysts consisting of DNA in the non-enzymatic portions, and RNA in the enzymatic core of hammerhead type ribozymes. Substitutions of DNA for RNA in the various stems of a hammerhead ribozyme have been analyzed in vitro for kinetic efficiency. One of the chimeric ribozymes used in this study, which harbors 24 bases of DNA capable of base-pairing interactions with an HIV-1 gag target, but maintains RNA in the catalytic center and in stem-loop II, has a sixfold greater kcat value than the all RNA counterpart. This increased activity appears to be the direct result of

enhanced product dissociation. Interestingly, a chimeric **ribozyme** in which stem-loop II (which divides the catalytic core) is comprised of DNA, exhibited a marked reduction in cleavage activity, suggesting that DNA in this region of the **ribozyme** can impart a negative effect on the catalytic function of the **ribozyme**. DNA-RNA chimeric **ribozymes** transfected by cationic **liposomes** into human T-lymphocytes are more stable than their all-RNA counterparts. Enhanced catalytic turnover and stability in the absence of a significant effect on Km make chimeric **ribozymes** favorable candidates for therapeutic agents.

4/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07248396 92172552 PMID: 1540406

Ribozymes as anti-HIV-1 therapeutic agents: principles, applications, and problems.

Rossi J J; Elkins D; Zaia J A; Sullivan S
Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

AIDS research and human retroviruses (UNITED STATES) Feb 1992, 8

(2) p183-9, ISSN 0889-2229 Journal Code: 8709376
Contract/Grant No.: AI25959; AI; NIAID; AI29329; AI; NIAID
Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

An emerging strategy in the treatment of viral infections is the use of antisense DNA or RNA to pair with, and block expression of viral transcripts. RNA, in addition to being an informational molecule, can also possess enzymatic activity. Thus, by combining anti-sense and enzymatic functions into a single transcript, it is now possible to design catalytic RNAs, or **ribozymes**, which can specifically pair with virtually any viral RNA, and cleave the phosphodiester backbone at a specified location, thereby functionally inactivating the viral RNA. In carrying out this cleavage, the **ribozyme** is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. There are several different catalytic motifs which possess enzymatic activity, and each one of these can be incorporated into an enzymatic antisense with site-specific cleavage capabilities. By focusing on one type of catalytic motif, the hammerhead, we describe the principles behind the development of **ribozymes** as transacting, site-specific ribonucleases, several applications of **ribozymes** in functional destruction of target RNAs, as well as several of the problems confronting their use. We also describe a **liposome** delivery system which facilitates intracellular inclusion of **ribozymes**, and may provide a means for therapeutic delivery of **ribozymes** to HIV-1 infected cells.

4/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07245871 92167261 PMID: 1538398

Preformed **ribozyme** destroys tumour necrosis factor mRNA in human cells.

Sioud M; Natvig J B; Forre O
Institute of Immunology and Rheumatology, University of Oslo, Norway.
Journal of molecular biology (ENGLAND) Feb 20 1992, 223 (4)
p831-5, ISSN 0022-2836 Journal Code: 2985088R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Maintaining RNA stability is a major problem in the delivery of preformed

inhibitory RNA to target cells. In this study, we delivered a hammerhead ribozyme directed against tumour necrosis factor alpha into human promyelocytic leukaemia cells by cationic liposome-mediated transfection. Delivering a ribozyme in this manner reduced by 90% and 85% tumour necrosis factor alpha mRNA and protein, respectively. A modified ribozyme with a bacteriophage T7 transcription terminator at its 3' end was more stable than one lacking this sequence. This indicates that ribozyme stability can be improved by the addition of terminal sequences expected to protect against cellular nucleases.

4/3,AB/16 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

11546689 BIOSIS NO.: 199800328021
Antisense molecules and ribozymes: Medical applications.
BOOK TITLE: Practical Approach Series; Antisense technology
AUTHOR: Strauss Michael(a)
BOOK AUTHOR/EDITOR: Lichtenstein C; Nellen W: Eds
AUTHOR ADDRESS: (a)Dep. Mol. Cell Biol., Humbolt-Univ., Max Debrueck Cent.
Mol. Med., Robert-Roessle-Str. 10, D-131**Germany
JOURNAL: Practical Approach Series 185p221-239 1997
BOOK PUBLISHER: Oxford University Press, Walton Street, Oxford OX2 6DP,
England
Oxford University Press, Inc., 198 Madison Avenue, New
York, New York 10016, USA
ISSN: 0957-025X ISBN: 0-19-963584-6 (cloth); 0-19-963583-8 (paper)
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English

1997

4/3,AB/17 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

10875038 BIOSIS NO.: 199799496183
Liposomes as drug and gene carriers.
AUTHOR: Lasic D D
AUTHOR ADDRESS: 7512 Birkdale Drive, Newark, CA 94560**USA
JOURNAL: Abstracts of Papers American Chemical Society 213 (1-3):pBIOT 1
1997
CONFERENCE/MEETING: 213th National Meeting of the American Chemical Society
San Francisco, California, USA April 13-17, 1997
ISSN: 0065-7727
RECORD TYPE: Citation
LANGUAGE: English

1997

4/3,AB/18 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

10754509 BIOSIS NO.: 199799375654
Targeted therapy for viral hepatitis.
AUTHOR: Schuster Martin J; Wu George Y
AUTHOR ADDRESS: Dep. Med., Div. Gastroenterology-Hepatology, Univ. Conn.
Sch. Med., Farmington, CT 06030-1845**USA
JOURNAL: Drugs of Today 32 (8):p653-661 1996
ISSN: 0025-7656
DOCUMENT TYPE: Literature Review

RECORD TYPE: Citation

LANGUAGE: English

1996

4/3,AB/19 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

10346289 BIOSIS NO.: 199698801207

Gene therapy: Basic concepts and recent advances.

AUTHOR: Sokol Deborah L; Gewirtz Alan M(a)

AUTHOR ADDRESS: (a)Dep. Pathol. Lab. Med., Univ. Pennsylvania Sch. Med.,
Philadelphia, PA 19104**USA

JOURNAL: Critical Reviews in Eukaryotic Gene Expression 6 (1):p29-57

1996

ISSN: 1045-4403

DOCUMENT TYPE: Literature Review

RECORD TYPE: Citation

LANGUAGE: English

1996

4/3,AB/20 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

09749082 BIOSIS NO.: 199598204000

Ribozyme and mRNA delivery using cationic liposomes.

AUTHOR: Malone Robert W

AUTHOR ADDRESS: Gene Therapy Program, Dep. Med. Pathol., Univ. Calif.,
Davis, CA 95616**USA

JOURNAL: Journal of Cellular Biochemistry Supplement 0 (19A):p206

1995

CONFERENCE/MEETING: Keystone Symposium on Ribozymes: Basic Science and
Therapeutic Applications Breckenridge, Colorado, USA January 15-21, 1995

ISSN: 0733-1959

RECORD TYPE: Citation

LANGUAGE: English

1995

4/3,AB/21 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

08984679 BIOSIS NO.: 199396136180

Liposome-mediated uptake of ribozymes.

AUTHOR: Sullivan Sean M

AUTHOR ADDRESS: Ribozyme Pharmaceuticals, Inc., P.O. Box 17280, Boulder,
CO 80308**USA

JOURNAL: Methods (Orlando) 5 (1):p61-66 1993

ISSN: 1046-2023

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: This article describes the necessary procedures used to deliver RNA to cells by liposomes. Ribozymes longer than 50 nucleotides in length were trapped routinely by these procedures. Larger ribozymes (140 nucleotides in length) and small plasmids (greater than 5 kb) can also be delivered by these procedures. Methods for liposome preparation, screening cell uptake, and subcellular fractionation are described.

1993

4/3,AB/22 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

08776530 BIOSIS NO.: 199395065881
Construction of several kinds of **ribozymes** and their reactivities.
AUTHOR: Nishikawa Satoshi; Taira Kazunari
AUTHOR ADDRESS: Fermentation Res. Inst., Higashi 1-1-3, Tsukuba, Ibaraki
305**Japan
JOURNAL: Report of the Fermentation Research Institute (Yatabe) 0 (75) :p
69-85 1992
ISSN: 0368-5365
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Japanese; Non-English
SUMMARY LANGUAGE: Japanese; English

ABSTRACT: Since the revolutionary discovery of the first **ribozyme** a decade ago, the list of RNA molecules with catalytic activity is growing. In this report, we will focus on two types of **ribozymes**, namely, hammerhead-type and HDV (hepatitis delta virus) **ribozymes**. Hammerhead-type **ribozymes** are potentially powerful agents for suppressing intracellular gene expression. There are basically two ways to introduce hammerhead and other types of **ribozymes** into cells. One such technique is the drug delivery system (DDS) in which synthesized **ribozymes** are encapsulated in **liposomes** or other related compounds and delivered to target cells. In this methodology, it is important to use relatively stable **ribozymes**. We find that significant portions of hammerhead-type **ribozymes** can be replaced by DNA counterparts possessing phosphorothioate linkages, without sacrificing catalytic activities. Moreover, the resulting chimeric thio-DNA/RNA **ribozymes** are more stable than all RNA **ribozymes** against nucleases. Another way to introduce **ribozymes** into cells is by transcription from the corresponding DNA template. When **ribozymes** are to be used as anti-HIV agents, it is better to target several sites simultaneously, since HIV has genetic variability. We have thus developed an expression vector, by combining cis- and trans-acting **ribozymes**, with which such a multiple targeting becomes possible. Finally, mutagenesis analyses on HDV **ribozymes** enabled us to identify two indispensable bases, G726 and C763, which are located in functionally important single-stranded regions.

1992

4/3,AB/23 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

08640386 BIOSIS NO.: 199345058461
Liposome uptake by human primary lymphoblasts.
AUTHOR: Sullivan S(a); Ayers D(a); Campbell T; Kuritzkes D; Sherrer J(a);
Schooley R
AUTHOR ADDRESS: (a)Ribozyne Pharmaceuticals Inc., Boulder, CO**USA

JOURNAL: Journal of Cellular Biochemistry Supplement 0 (17 PART E) :p23

1993

CONFERENCE/MEETING: Keystone Symposium on Frontiers in HIV Pathogenesis
Albuquerque, New Mexico, USA March 29-April 4, 1993

ISSN: 0733-1959

RECORD TYPE: Citation

LANGUAGE: English

? s ribozyme and vegf
5179 RIBOZYME
14719 VEGF
S1 33 RIBOZYME AND VEGF
? rd
...completed examining records
S2 26 RD (unique items)
? t s2/3,ab/all

2/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155: MEDLINE(R)

14293602 22388811 PMID: 12500395

Experimental study of anti-**VEGF** hairpin **ribozyme** gene inhibiting expression of **VEGF** and proliferation of ovarian cancer cells]

Yan Rui-lan; Qian Xin-hua; Xin Xiao-yan; Jin Ming; Hui Hong-xiang; Wang De-tang; Wang Jian; et al

Department of Gynecology and Obstetrics, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, P. R. China. yanrl@fmmu.edu.cn

Ai Zheng (China) Jan 2002, 21 (1) p39-44, ISSN 1000-467X

Journal Code: 9424852

Document type: Journal Article ; English Abstract

Languages: CHINESE

Main Citation Owner: NLM

Record type: Completed

BACKGROUND & OBJECTIVE: Growth of solid tumor metastases are critically dependent on angiogenesis. Vascular endothelial growth factor (**VEGF**), also known as vascular permeability factor, has been identified as one of the most potent inducers of tumor associated angiogenesis, studies have shown that **VEGF** plays an important role in angiogenesis which is associated with epithelial ovarian cancer. Until now, many strategies for gene therapy have been developed. Among them is **Ribozyme**-based therapeutics for cancer which might be devised to inhibit tumor growth or prevent metastases. Angiogenesis is required for sustained tumor growth, making the **VEGF** pathway another promising target for either small molecule or nucleic acid-based therapeutics. Little is known about the role of **VEGF** in ovarian tumorigenecity. We propose to block the autocrine and/or paracrine pathway of **VEGF** in ovarian cancer using anti-**VEGF** hairpin **ribozyme** gene to see whether the growth of tumor cells could be inhibited and to further exploit its mechanisms. **METHODS:** Anti-**VEGF** hairpin **ribozyme** gene eukaryotic expression vector was introduced into ovarian cancer SKOV3 cells by lipofectin mediation and positive clones were screened by G418; **Ribozyme** expression was confirmed by RNA dot blot; The **VEGF** expression of SKOV3 cells before or after transfection were detected by immunohistochemical and immunofluorescence and flow cytometer immunofluorescence methods, MTT, colony forming, soft agar colony forming, and FCM were used to observe the effect of proliferation to ovarian cancer cells. **RESULTS:** **VEGF** expression decreased distinctly in SKOV3-RZ cells. The growth of transfected SKOV3-RZ cells were slower, The average colony forming efficiency and soft agar colony forming efficiency of SKOV3-RZ cells(12.7 +/- 1.4 and 9.4 +/- 2.0, respectively) decreased distinctly (P < 0.001). The SKOV3-RZ cells of G1 stage increased(P < 0.01), the SKOV3-RZ cells of S stage were reduced(P < 0.01). **CONCLUSIONS:** Anti-**VEGF** hairpin **ribozyme** gene can inhibit the proliferation of ovarian cancer SKOV3 cells. This provides a experimental basis for cure human ovarian cancer with antiangiogenesis method.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155: MEDLINE(R)

14194916 22377099 PMID: 12487926

Experimental study of anti-vascular endothelial growth factor hairpin ribozyme gene inhibiting growth of xenografted tumor of ovarian cancer cells.

Yan Ruilan; Xin Xiaoyan; Jin Ming; Hui Hongxiang; Wang Jian; Wang Detang; et al

Department of Gynecology and Obstetrics, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China.

Zhonghua fu chan ke za zhi (China) Nov 2002, 37 (11) p683-6, ISSN 0529-567X Journal Code: 16210370R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

OBJECTIVE: We propose to block the autocrine and (or) paracrine pathway of vascular endothelial growth factor (VEGF) in ovarian cancer by using anti-VEGF hairpin ribozyme gene to see whether the growth of tumor cells could be inhibited and to further exploit its mechanisms.

METHODS: Anti-VEGF hairpin ribozyme gene eukaryotic expression vector was introduced into ovarian cancer cells SKOV(3) by using lipofectin mediation and positive clones were screened by gentycin (G418); the ribozyme (RZ) expression was confirmed by RNA dot blot; the VEGF expression of SKOV(3) cells before or after transfection were detected by reverse transcription polymerase chain reaction (RT-PCR) method, transmission electron microscopy demonstrated the morphologic changes of ribozyme -generating SKOV(3) cells; the growth of transfected cells in nude mice were detected. RESULTS: VEGF expression was decreased distinctly in SKOV(3) cells transfected by RZ (SKOV(3)-RZ) cells. Apoptosis cells were observed; the formation rate and growth speed of xenografted tumor slowed down. CONCLUSIONS: Anti-VEGF hairpin ribozyme gene can inhibit the expression of VEGF mRNA and VEGF in SKOV(3) cells. The growth of xenografted tumor in nude mice was inhibited by reduced angiogenesis. This provides an experimental basis for curing human ovarian cancer with antiangiogenesis method.

2/3,AB/3 (Item 3 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

14096730 22057963 PMID: 12063553

Ribozyme mediated suppression of vascular endothelial growth factor gene expression enhances matrix metalloproteinase 1 expression in a human hepatocellular carcinoma cell line.

Kamochi Junichiro; Tokunaga Tetsuji; Morino Fumitoshi; Nagata Junko; Tomii Yasushi; Abe Yoshiyuki; Hatanaka Hiroyuki; Kijima Hiroshi; Yamazaki Hitoshi; Watanabe Norihito; Matsuzaki Shohei; Ueyama Yoshito; Nakamura Masato; et al

Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan.

International journal of oncology (Greece) Jul 2002, 21 (1) p81-4, ISSN 1019-6439 Journal Code: 9306042

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The levels of expression of various genes were altered in cellular transformants with manipulation of expression of single genes. Vascular endothelial growth factor A (VEGF-A) is a key molecule for tumor progression, although it is unclear how VEGF-A expression regulates various genes. Multiple gene expression levels were evaluated using cDNA arrays in a human hepatocellular carcinoma cell line (HLF) with suppression of the VEGF-A gene by anti-VEGF-A ribozyme (alphaVRz).

The ribozyme-mediated suppression of VEGF-A gene solely up-regulated matrix metalloproteinase 1 (MMP1) gene level in HLF/alphaVRz.

Levels of expression of other members of MMP family or tissue inhibitors of MMPs did not show any alteration. These results suggested that intracellular suppression of VEGF-A gene was specifically linked to up-regulation of MMP1 in human hepatocellular carcinoma cells.

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155: MEDLINE(R)

13981924 22257569 PMID: 12370751

Ribozyme mediated cleavage of cell-associated isoform of vascular endothelial growth factor inhibits liver metastasis of a pancreatic cancer cell line.

Tokunaga Tetsuji; Abe Yoshiyuki; Tsuchida Takashi; Hatanaka Hiroyuki; Oshika Yoshiro; Tomisawa Masashi; Yoshimura Masumi; Ohnishi Yasuyuki; Kijima Hiroshi; Yamazaki Hitoshi; Ueyama Yoshito; Nakamura Masato; et al
Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan.

International journal of oncology (Greece) Nov 2002, 21 (5) p1027-32
ISSN 1019-6439 Journal Code: 9306042

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

Stromal angiogenesis is an important factor for progression of malignant neoplasms. We used hammerhead ribozymes against vascular endothelial growth factor (VEGF) gene transcripts to down-regulate cell-associated VEGF189 isoform function in the pancreatic cancer cell line MIA PaCa2. MIA PaCa2 transfected with anti-VEGF189 ribozyme did not show any alteration of growth rate under tissue culture. When the transformants were subcutaneously transplanted, tumour volume of the ribozyme-transfected MIA PaCa2 xenografts was significantly smaller ($P<0.01$). No metastasis of MIA PaCa2 transfected with anti-VEGF189 was apparent, while disabled ribozyme-transfected MIA PaCa2 showed significant liver metastasis ($P<0.05$). These results suggested that VEGF189 plays an important role in growth and metastatic potential through alteration of angiogenic balance in cancer.

2/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155: MEDLINE(R)

11340592 21468612 PMID: 11094315

Ribozyme approach to downregulate vascular endothelial growth factor (VEGF) 189 expression in non-small cell lung cancer (NSCLC).

Oshika Y; Nakamura M; Tokunaga T; Ohnishi Y; Abe Y; Tsuchida T; Tomii Y; Kijima H; Yamazaki H; Ozeki Y; Tamaoki N; Ueyama Y
Department of Pathology, Tokai University School of Medicine, Isehara-shi, Kanagawa, Japan.

European journal of cancer (Oxford, England : 1990) (England) Dec 2000, 36 (18) p2390-6, ISSN 0959-8049 Journal Code: 9005373

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The aim of this study was to further clarify the role of the cell-associated isoform of vascular endothelial growth factor (VEGF189) on tumour growth and vascularity. Five isoforms of VEGF have been identified with different biological activities. VEGF121, VEGF145, VEGF165, VEGF189, VEGF206 are generated by alternative splicing. We used a hammerhead-type ribozyme (V189Rz) to suppress VEGF189 mRNA. The V189Rz specifically cleaved exon 6 of VEGF189 mRNA, but showed no activity against the VEGF121 or VEGF165 isoforms. The V189Rz was introduced into the human non-small cell lung cancer (NSCLC) cell line (OZ-6/VR). The

expression level of VEGF189 mRNA was decreased in the OZ-6/VR cells, while VEGF121 and 165 expression was unaltered. The OZ-6/VR cells xenotransplanted into nude mice showed markedly reduced vascularisation and growth, whereas the cell line did not show any decreased growth under tissue culture conditions. The OZ-6/VR cells (1×10^5 cells/mouse) formed no tumours, whereas the parental OZ-6 cells formed large tumours within 8 weeks. The specific suppression of VEGF189 by the **ribozyme** decreased vascularity and xenotransplantability of the lung cancer cell line. Thus, the cell-associated isoform of **VEGF**, VEGF189, might have a key role in stromal vascularisation and the growth of NSCLC xenografts *in vivo*.

2/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

11109175 21114650 PMID: 11177746

Angiozyme: a novel angiogenesis inhibitor.

Weng D E; Usman N

Department of Hematology and Oncology, The Cleveland Clinic Foundation,
9500 Euclid Avenue, T-40, Cleveland, OH 44195, USA. wengd@ccf.org

Current oncology reports (United States) Mar 2001, 3 (2) p141-6,

ISSN 1523-3790 Journal Code: 100888967

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Several inhibitors of angiogenesis are being developed for the treatment of cancer. One dominant strategy involves disruption of the vascular endothelial growth factor (**VEGF**) pathway by inhibition of the receptors for **VEGF**. Inhibition of the **VEGF** receptor activity can be accomplished using catalytic RNA molecules known as ribozymes, which downregulate **VEGF** receptor function by specifically cleaving the mRNAs for the primary **VEGF** receptors, Flt-1 and KDR. Significant inhibition of angiogenesis using ribozymes against both receptors has been demonstrated. In animal tumor models, antitumor effects are most pronounced with the anti-Flt-1 **ribozyme** known as Angiozyme (**Ribozyme Pharmaceuticals**, Boulder, CO). Extensive preclinical studies have demonstrated no significant toxicities. Clinical trials of Angiozyme are currently in progress for patients with advanced malignancy. Preliminary results demonstrate Angiozyme to be well tolerated, without significant side effects. Several phase II trials are underway for patients with advanced malignancy to test therapeutic efficacy.

2/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

11012485 21000746 PMID: 11185667

Pharmacokinetics and tolerability of an antiangiogenic **ribozyme** (ANGIOZYME) in healthy volunteers.

Sandberg J A; Parker V P; Blanchard K S; Sweedler D; Powell J A;
Kachensky A; Bellon L; Usman N; Rossing T; Borden E; Blatt L M
Ribozyme Pharmaceuticals, Inc., 2950 Wilderness Place, Boulder, Colorado
80301, USA.

Journal of clinical pharmacology (United States) Dec 2000, 40 (12 Pt
2) p1462-9, ISSN 0091-2700 Journal Code: 0366372

Document type: Clinical Trial; Journal Article; Randomized Controlled
Trial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The pharmacokinetics and tolerability of a chemically stabilized synthetic **ribozyme** (ANGIOZYME) targeting the Flt-1 **VEGF** receptor mRNA were evaluated in healthy volunteers. In a

placebo-controlled, single-dose escalation study, **ribozyme** was administered as a 4-hour i.v. infusion of 10 or 30 mg/m² or as a s.c. bolus of 20 mg/m². Peak **ribozyme** plasma concentrations of 1.5 and 3.8 micrograms/mL were observed after the 10 and 30 mg/m² i.v. infusions, respectively. When normalized to dose, AUC values as well as peak concentrations increased proportionally as the dose was increased from 10 to 30 mg/m². Peak concentrations of 0.9 microgram/mL were observed approximately 3.25 hours after a 20 mg/m² s.c. bolus of **ribozyme**. The dose-normalized AUCs obtained after s.c. dosing were compared to the mean dose-normalized AUC after i.v. dosing to estimate an absolute s.c. bioavailability (f) of approximately 69%. An average elimination half-life of 28 to 40 minutes was observed after i.v. administration, which increased to 209 minutes after s.c. administration. Only 4 of 12 reported adverse events were possibly related to administration of **ribozyme** (headache and somnolence). Thus, **ribozyme** administration was well tolerated after a single 4-hour i.v. infusion of up to 30 mg/m² or a single s.c. bolus of 20 mg/m².

2/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

10866722 20411133 PMID: 10952790

Ribozyme-mediated inactivation of mutant K-ras oncogene in a colon cancer cell line.

Tokunaga T; Tsuchida T; Kijima H; Okamoto K; Oshika Y; Sawa N; Ohnishi Y; Yamazaki H; Miura S; Ueyama Y; Nakamura M

Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, 259-1193, Japan.

British journal of cancer (SCOTLAND) Sep 2000, 83 (6) p833-9, ISSN 0007-0920 Journal Code: 0370635

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Mutation of c-K-ras oncogene is an important step in progression of colon cancer. We used a hammerhead **ribozyme** (KrasRz) against mutated K-ras gene transcripts (codon 12, GTT) to inactivate mutant K-ras function in the colon cancer cell line SW480, harbouring a mutant K-ras gene. The beta-actin promoter-driven KrasRz sequence (pHbeta/KrasRz) was introduced into these cells (SW480/KrasRz), and we evaluated its effects on growth of the colon cancer. The gene expression of angiogenesis-related molecules (vascular endothelial growth factor and thrombospondin) was also estimated in SW480/KrasRz. KrasRz specifically and efficiently cleaved the mutant K-ras mRNA but not wild-type mRNA in vitro. SW480/KrasRz showed decreased growth rate under tissue culture conditions ($P < 0.01$, Dunnett's test). The xenotransplantability of SW480/KrasRz (XeSW480/KrasRz) was significantly decreased in nude mice ($P < 0.05$, Fisher's exact test). Tumour volume of the xenografts XeSW480/KrasRz was significantly smaller than that of XeSW480/DisKrasRz ($P < 0.01$, Dunnett's test). Gene expression of VEGF was suppressed in SW480/KrasRz, while TSP1 gene expression was enhanced. The SW480/KrasRz cells showed apoptosis-related features including nuclear condensation and DNA fragmentation. These results suggested that the hammerhead **ribozyme**-mediated inactivation of the mutated K-ras mRNA induced growth suppression, apoptosis and alteration of angiogenic factor expression. Copyright 2000 Cancer Research Campaign.

2/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

10855672 20398618 PMID: 10938389

Hammerhead **ribozyme** specifically inhibits vascular endothelial growth factor gene expression in a human hepatocellular carcinoma cell

line.

Morino F; Tokunaga T; Tsuchida T; Handa A; Nagata J; Tomii Y; Kijima H; Yamazaki H; Watanabe N; Matsuzaki S; Ueyama Y; Nakamura M

Department of Pathology, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, Japan.

International journal of oncology (GREECE) Sep 2000, 17 (3) p495-9,
ISSN 1019-6439 Journal Code: 9306042

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hammerhead-type ribozymes are often utilized to suppress the expression of target genes. We evaluated the efficacy of an anti-vascular endothelial growth factor (**VEGF**) hammerhead-type **ribozyme** against GUC at exon 1 of the **VEGF** gene in a cell-free system (in vitro) as well as in the hepatocellular carcinoma cell line HLF (in vivo). The anti-**VEGF** **ribozyme** (alphaVRz) specifically cleaved synthetic **VEGF** RNA substrate, but not other triplet sequences of **VEGF** RNA substrate in vitro. When the alphaVRz was introduced into HLF cells, the **ribozyme** suppressed not only **VEGF** mRNA level but also that of **VEGF** protein. These results suggest that this **ribozyme** selectively inhibits **VEGF** gene expression in human hepatocellular carcinoma cells.

2/3,AB/10 (Item 10 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

10824576 20361487 PMID: 10905552

Acute toxicology and pharmacokinetic assessment of a **ribozyme** (**ANGIOZYME**) targeting vascular endothelial growth factor receptor mRNA in the cynomolgus monkey.

Sandberg J A; Sproul C D; Blanchard K S; Bellon L; Sweedler D; Powell J A ; Caputo F A; Kornbrust D J; Parker V P; Parry T J; Blatt L M
Ribozyne Pharmaceuticals, Inc., Boulder, CO 80301, USA.

Antisense & nucleic acid drug development (UNITED STATES) Jun 2000, 10 (3) p153-62, ISSN 1087-2906 Journal Code: 9606142

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The potential acute toxicity of a **ribozyme** (**ANGIOZYME**) targeting the flt-1 vascular endothelial growth factor (**VEGF**) receptor mRNA was evaluated in cynomolgus monkeys following i.v. infusion or s.c. injection. **ANGIOZYME** was administered as a 4-hour i.v. infusion at doses of 10, 30, or 100 mg/kg or a s.c. bolus at 100 mg/kg. End points included blood pressure, electrocardiogram (ECG), clinical chemistry, hematology, complement factors, coagulation parameters, and **ribozyme** plasma concentrations. **ANGIOZYME** was well tolerated, with no drug-associated morbidity or mortality. There was no clear evidence of **ANGIOZYME**-related adverse effects in this study. Slight increases in spleen weight and lymphoid hyperplasia were observed in several animals. However, these changes were not dose dependent. Steady-state concentrations of **ANGIOZYME** were achieved during the 4-hour infusion of 10, 30, or 100 mg/kg. Dose-dependent elimination of **ANGIOZYME** was observed, with faster clearance at the two highest doses. **ANGIOZYME** was slowly absorbed after s.c. administration, resulting in steady-state concentrations for the 9-hour sampling period. Monkeys in this toxicology study received significant plasma **ANGIOZYME** exposure by both the s.c. and i.v. routes.

2/3,AB/11 (Item 11 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

10739203 20273384 PMID: 10815937

Antitumor and antimetastatic activity of ribozymes targeting the messenger RNA of vascular endothelial growth factor receptors.

Pavco P A; Bouhana K S; Gallegos A M; Agrawal A; Blanchard K S; Grimm S L; Jensen K L; Andrews L E; Wincott F E; Pitot P A; Tressler R J; Cushman C; Reynolds M A; Parry T J

Ribozyme Pharmaceuticals Inc, Boulder, Colorado 80301, USA. pavco@rpi.com
Clinical cancer research : an official journal of the American Association for Cancer Research (UNITED STATES) May 2000, 6 (5) p2094-103, ISSN 1078-0432 Journal Code: 9502500

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Chemically stabilized hammerhead ribozymes are nuclease-resistant, RNA-based oligonucleotides that selectively bind and cleave specific target RNAs. Due to their potential for specifically inhibiting gene expression, ribozymes are being investigated for therapeutic applications as well as for the elucidation of gene function. In particular, we have investigated ribozymes that target the mRNA of the vascular endothelial growth factor (**VEGF**) receptors because **VEGF** signaling is an important mediator of tumor angiogenesis and metastasis. Here we report pharmacodynamic studies testing anti-Flt-1 (VEGFR-1) and anti-KDR (VEGFR-2) ribozymes in animal models of solid tumor growth and metastasis. Ribozymes targeting either Flt-1 or KDR significantly inhibited primary tumor growth in a highly metastatic variant of Lewis lung carcinoma. However, only treatment with the anti-Flt-1 **ribozyme** resulted in a statistically significant and dose-dependent inhibition of lung metastasis in this model. The anti-Flt-1 **ribozyme** was then tested in a xenograft model of human metastatic colorectal cancer in which significant inhibition of liver metastasis was observed. Taken together, these data represent the first demonstration that synthetic ribozymes targeting **VEGF** receptor mRNA reduced the growth and metastasis of solid tumors *in vivo*.

2/3,AB/12 (Item 12 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

10512165 20036191 PMID: 10571413

Scatter factor/hepatocyte growth factor (SF/HGF) content and function in human gliomas.

Lamszus K; Laterra J; Westphal M; Rosen E M

Department of Neuropathology, University Hospital Eppendorf, Hamburg, Germany. lamszus@plexus.uke.uni-hamburg.de

International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience (ENGLAND) Aug-Oct 1999, 17 (5-6) p517-30, ISSN 0736-5748

Journal Code: 8401784

Contract/Grant No.: NS32148; NS; NINDS

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Scatter factor/hepatocyte growth factor (SF/HGF) is a pleiotrophic cytokine that stimulates motility and invasion of several cancer cell types and induces angiogenesis. Its receptor MET is a transmembrane tyrosine kinase encoded by the C-MET proto-oncogene. To assess the potential relevance of SF/HGF in gliomas we performed functional studies *in vivo* and *in vitro*, expression analyses and correlative studies. We showed that both SF/HGF and MET are expressed in gliomas *in vivo* and are upregulated during transition from low grade to malignant glioma. When SF/HGF cDNA was transfected into glioma cells that expressed the MET receptor the cells formed considerably larger and more vascularized intracranial tumors *in vivo* than SF/HGF negative control clones. In other glioma cells, which

constitutively expressed both SF/HGF and MET, we abolished SF/HGF expression by antisense **ribozyme**-targeting, which led to a significant decrease in tumorigenicity and tumor growth. In vitro SF/HGF strongly stimulated glioma cell motility and to a lesser degree proliferation. SF/HGF also strongly increased endothelial cell motility in vitro and extracts of tumors derived from SF/HGF-transfected glioma cells were more mitogenic for endothelial cells and more angiogenic in the rat cornea angiogenesis assay than extracts from control tumors. In a three-dimensional in vitro angiogenesis assay basic fibroblast growth factor (bFGF) was found to synergize with either SF/HGF or vascular endothelial growth factor (**VEGF**) in inducing endothelial capillary-like tubes, whereas neither SF/HGF nor **VEGF** alone or in combination were effective. Interestingly, while both **VEGF** and SF/HGF levels appeared to be increased in malignant gliomas compared with low grade ones, this was not the case for bFGF of which biologically relevant levels were already present in low grade gliomas. It thus seems that bFGF alone is insufficient to induce angiogenesis in gliomas but may act synergistically with either **VEGF** and/or SF/HGF when these become upregulated during malignant progression. In conclusion, we showed that SF/HGF may contribute to glioma progression by stimulating tumor invasiveness, proliferation and neovascularization.

2/3,AB/13 (Item 13 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

10381754 99362105 PMID: 10435752

Pharmacokinetics of an antiangiogenic **ribozyme** (ANGIOZYME) in the mouse.

Sandberg J A; Bouhana K S; Gallegos A M; Agrawal A B; Grimm S L; Wincott F E; Reynolds M A; Pavco P A; Parry T J

Ribozyme Pharmaceuticals, Inc., Boulder, CO 80301, USA.

Antisense & nucleic acid drug development (UNITED STATES) Jun 1999, 9

(3) p271-7, ISSN 1087-2906 Journal Code: 9606142

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vascular endothelial growth factor (**VEGF**) is a growth factor that contributes to the angiogenesis of developing tumors. To interfere with the action of **VEGF**, a nuclease-stabilized **ribozyme**, ANGIOZYME, has been developed against **VEGF** receptor subtype Flt-1 mRNA. To determine which routes of administration would be useful for systemic delivery of this **ribozyme**, a dose of 30 mg/kg [³²P]ANGIOZYME was administered as an i.v., i.p., or s.c. bolus. Concentrations of ANGIOZYME in plasma, femur, kidney, liver, and lung were examined. ANGIOZYME was well absorbed after i.p. (90%) or s.c. administration (77%), with peak plasma concentrations occurring 30 minutes after dosing. Total body clearance after a single dose of 30 mg/kg ANGIOZYME was 20 ml/min/kg, and the elimination half-life was 33 minutes. The apparent volume of distribution at steady-state ranged from 0.5 to 1.3 L/kg. ANGIOZYME was detected in the four tissues examined through the 3 hour sampling period after i.v. or i.p. administration. After s.c. administration, ANGIOZYME was detected in femur, kidney, and lung but not in the liver. The highest concentrations of ANGIOZYME were found in kidney and femur with all three routes. Because of the rapid and extensive absorption after extravascular injections, either i.p. or s.c. administration could be considered for use in pharmacodynamic studies examining the effects of ANGIOZYME or other ribozymes with similar chemical modifications.

2/3,AB/14 (Item 14 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

09841723 98256490 PMID: 9592205

A novel approach to glioma gene therapy: down-regulation of the vascular endothelial growth factor in glioma cells using ribozymes.

Ke L D; Fueyo J; Chen X; Steck P A; Shi Y X; Im S A; Yung W K
Department of Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA.

International journal of oncology (GREECE) Jun 1998, 12 (6) p1391-6,
ISSN 1019-6439 Journal Code: 9306042

Contract/Grant No.: CA51148; CA; NCI; CA55261; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Glioblastoma multiforme is one of the most highly vascularized solid neoplasms, therefore treatments that target neovascularization process would be of great clinical importance. Studies of glioblastoma angiogenesis have revealed that expression of the vascular endothelial growth factor (**VEGF**) is up-regulated in these tumors. Previous reports have shown that down-regulation of **VEGF** correlates with modification in the glioma growth. To examine this phenomenon further, in this study we constructed two hammerhead ribozymes (RZI and RZII) to target the 5' common region of **VEGF** mRNA. Both ribozymes exhibited site-specific cleavage to a 318-nucleotide **VEGF** transcript and showed a high digestion efficiency in vitro (65-95%). After the transfection of glioma cells with two expression vectors carrying the **ribozyme** sequence, Northern blot analyses detected high levels of **ribozyme** expression. Treatment of the glioma cells with the ribozymes resulted in a reduction in **VEGF** mRNA in six of eight clones. Furthermore, the anti-**VEGF** effect was confirmed at protein level. Thus, enzyme-linked immunoabsorbent analyses (ELISA) showed a >70% reduction in the VEGF165 expression level. These results indicate that hammerhead ribozymes may be useful in down-regulating **VEGF** expression and suggest that anti-**VEGF** strategies may be used to potentiate other gene therapies targeting tumor suppressor genes.

2/3,AB/15 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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14067873 BIOSIS NO.: 200300061902

Inhibitors of the vascular endothelial growth factor receptor.

AUTHOR: Rosen Lee S(a)

AUTHOR ADDRESS: (a)Cancer Institute Medical Group, St. John's Health Center, 2001 Santa Monica Boulevard, Suite 560W, Santa Monica, CA, 90404, USA**USA E-Mail: rosenl@jwci.org

JOURNAL: Hematology-Oncology Clinics of North America 16 (5):p1173-1187

October 2002 2002

MEDIUM: print

ISSN: 0889-8588

DOCUMENT TYPE: Literature Review

RECORD TYPE: Citation

LANGUAGE: English

2002

2/3,AB/16 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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13409495 BIOSIS NO.: 200200038316

Anti-**VEGF** hammerhead-type **ribozyme** specifically decreased **VEGF** gene expression and protein level in a human hepatocellular carcinoma cell line.

AUTHOR: Morino Fumitoshi(a); Tokunaga Tetsuji; Kamochi Junichiro; Ueyama

Yoshito; Nakamura Masato; Miyachi Hayato; Matsuzaki Shohei
AUTHOR ADDRESS: (a)Department of Clinical Pathology, Isehara**Japan
JOURNAL: Proceedings of the American Association for Cancer Research Annual
Meeting 42p821 March, 2001
MEDIUM: print
CONFERENCE/MEETING: 92nd Annual Meeting of the American Association for
Cancer Research New Orleans, LA, USA March 24-28, 2001
ISSN: 0197-016X
RECORD TYPE: Citation
LANGUAGE: English
2001

2/3,AB/17 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

12811366 BIOSIS NO.: 200100018515
Pharmacokinetics and tolerability of an antiangiogenic **ribozyme**
(ANGIOZYMETM) in healthy volunteers.
AUTHOR: Sandberg Jennifer A(a); Parker Vann P; Blanchard Karin S; Sweedler
David; Powell James A; Kachensky Arlee; Bellon Laurent; Usman Nassim;
Rossing Thomas; Borden Ernest; Blatt Lawrence M
AUTHOR ADDRESS: (a)Ribozyme Pharmaceuticals, Inc., 2950 Wilderness Place,
Boulder, CO, 80301**USA
JOURNAL: Journal of Clinical Pharmacology 40 (12 Part 2):p1462-1469
December, 2000
MEDIUM: print
ISSN: 0091-2700
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The pharmacokinetics and tolerability of a chemically stabilized synthetic **ribozyme** (ANGIOZYMETM) targeting the Flt-1 VEGF receptor mRNA were evaluated in healthy volunteers. In a placebo-controlled, single-dose escalation study, **ribozyme** was administered as a 4-hour IV infusion of 10 or 30 mg/m² or as a SC bolus of 20 mg/m². Peak **ribozyme** plasma concentrations of 1.5 and 3.8 mug/mL were observed after the 10 and 30 mg/m² IV infusions, respectively. When normalized to dose, AUC values as well as peak concentrations increased proportionally as the dose was increased from 10 to 30 mg/m². Peak concentrations of 0.9 mug/mL were observed approximately 3.25 hours after a 20 mg/m² SC bolus of **ribozyme**. The dose-normalized AUCs obtained after SC dosing were compared to the mean dose-normalized AUC after IV dosing to estimate an absolute SC bioavailability (f) of approximately 69%. An average elimination half-life of 28 to 40 minutes was observed after IV administration, which increased to 209 minutes after SC administration. Only 4 of 12 reported adverse events were possibly related to administration of **ribozyme** (headache and somnolence). Thus, **ribozyme** administration was well tolerated after a single 4-hour IV infusion of up to 30 mg/m² or a single SC bolus of 20 mg/m².

2000

2/3,AB/18 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

12641508 BIOSIS NO.: 200000395010
Acute toxicology and pharmacokinetic assessment of a **ribozyme**

(ANGIOZYMETM) targeting vascular endothelial growth factor receptor mRNA in the cynomolgus monkey.

AUTHOR: Sandberg Jennifer A(a); Sproul Christopher D; Blanchard Karin S; Bellon Laurent; Sweedler David; Powell James A; Caputo Florence A; Kornbrust Douglas J; Parker Vann P; Parry Tom J; Blatt Lawrence M

AUTHOR ADDRESS: (a)Ribozyne Pharmaceuticals, Inc. (RPI), 2950 Wilderness Place, Boulder, CO, 80301**USA

JOURNAL: Antisense & Nucleic Acid Drug Development 10 (3):p153-162 June, 2000

MEDIUM: print

ISSN: 1087-2906

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: The potential acute toxicity of a **ribozyme** (ANGIOZYMETM) targeting the flt-1 vascular endothelial growth factor (**VEGF**) receptor mRNA was evaluated in cynomolgus monkeys following i.v. infusion or s.c. injection. ANGIOZYME was administered as a 4-hour i.v. infusion at doses of 10, 30, or 100 mg/kg or a s.c. bolus at 100 mg/kg. End points included blood pressure, electrocardiogram (ECG), clinical chemistry, hematology, complement factors, coagulation parameters, and **ribozyme** plasma concentrations. ANGIOZYME was well tolerated, with no drug-associated morbidity or mortality. There was no clear evidence of ANGIOZYME-related adverse effects in this study. Slight increases in spleen weight and lymphoid hyperplasia were observed in several animals. However, these changes were not dose dependent. Steady-state concentrations of ANGIOZYME were achieved during the 4-hour infusion of 10, 30, or 100 mg/kg. Dose-dependent elimination of ANGIOZYME was observed, with faster clearance at the two highest doses. ANGIOZYME was slowly absorbed after s.c. administration, resulting in steady-state concentrations for the 9-hour sampling period. Monkeys in this toxicology study received significant plasma ANGIOZYME exposure by both the s.c. and i.v. routes.

2000

2/3,AB/19 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

12498877 BIOSIS NO.: 200000252379
Ribozyme against **VEGF** expression inhibited tumor growth in nude mice.

AUTHOR: Ke Li Dao(a); Shi Yue-Xi(a); Yung WK Alfred(a)

AUTHOR ADDRESS: (a)M D Anderson Cancer Ctr, Houston, TX**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41):p568 March, 2000

CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

2000

2/3,AB/20 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2003 BIOSIS. All rts. reserv.

12484851 BIOSIS NO.: 200000238353

Evaluation of the toxicity and pharmacokinetics of a **ribozyme** targeting **VEGF** receptor Flt-1 mRNA (ANGIOZYMETM) in mice and monkeys.

AUTHOR: Sandberg J A(a); Ivens I A; Leibbrandt M E I; Samara E; Wolfgang G H I; Todd M D

AUTHOR ADDRESS: (a)Chiron Corp, Emeryville, CA**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41):p327 March, 2000

CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

2000

2/3,AB/21 (Item 7 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

12128203 BIOSIS NO.: 199900423052

Bioactivity of anti-angiogenic ribozymes targeting Flt-1 and KDR mRNA.

AUTHOR: Parry Tom J; Cushman Cynthia; Gallegos Anna M; Agrawal Arun B; Richardson Michele; Andrews Lori E; Maloney Lara; Mokler Victor R; Wincott Francine E; Pavco Pamela A(a)

AUTHOR ADDRESS: (a)Ribozyne Pharmaceuticals, Inc., 2950 Wilderness Place, Boulder, CO, 80301**USA

JOURNAL: Nucleic Acids Research 27 (13):p2569-2577 July 1, 1999

ISSN: 0305-1048

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: Vascular endothelial growth factor (**VEGF**) and its receptors Flt-1 and KDR play important roles in physiological and pathological angiogenesis. Ribozymes that target the **VEGF** receptor mRNAs were developed and their biological activities in cell culture and an animal model were assessed. Ribozymes targeting Flt-1 or KDR mRNA sites reduced **VEGF**-induced proliferation of cultured human vascular endothelial cells and specifically lowered the level of Flt-1 or KDR mRNA present in the cells. Anti-Flt-1 and KDR ribozymes also exhibited anti-angiogenic activity in a rat corneal pocket assay of **VEGF**-induced angiogenesis. This report illustrates the anti-angiogenic potential of these ribozymes as well as their value in studying **VEGF** receptor function in normal and pathophysiologic states.

1999

2/3,AB/22 (Item 8 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)

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12107753 BIOSIS NO.: 199900402602

Pharmacokinetics of an antiangiogenic **ribozyme** (ANGIOZYME(R)) in the mouse.

AUTHOR: Sandberg Jennifer A(a); Bouhana Karyn S; Gallegos Anna M; Agrawal Arun B; Grimm Susan L; Wincott Francine E; Reynolds Mark A; Pavco Pamela A; Parry Tom J

AUTHOR ADDRESS: (a)Ribozyne Pharmaceuticals, Inc., 2950 Wilderness Place, Boulder, CO, 80301**USA

JOURNAL: Antisense & Nucleic Acid Drug Development 9 (3):p271-277 June,

1999

ISSN: 1087-2906

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: Vascular endothelial growth factor (**VEGF**) is a growth factor that contributes to the angiogenesis of developing tumors. To interfere with the action of **VEGF**, a nuclease-stabilized **ribozyme**, **ANGIOZYME(R)**, has been developed against **VEGF** receptor subtype Flt-1 mRNA. To determine which routes of administration would be useful for systemic delivery of this **ribozyme**, a dose of 30 mg/kg (32P)**ANGIOZYME(R)** was administered as an i.v., i.p., or s.c. bolus. Concentrations of **ANGIOZYME(R)** in plasma, femur, kidney, liver, and lung were examined. **ANGIOZYME(R)** was well absorbed after i.p. (90%) or s.c. administration (77%), with peak plasma concentrations occurring 30 minutes after dosing. Total body clearance after a single dose of 30 mg/kg **ANGIOZYME(R)** was 20 ml/min/kg, and the elimination half-life was 33 minutes. The apparent volume of distribution at steady-state ranged from 0.5 to 1.3 L/kg. **ANGIOZYME(R)** was detected in the four tissues examined through the 3 hour sampling period after i.v. or i.p. administration. After s.c. administration, **ANGIOZYME(R)** was detected in femur, kidney, and lung but not in the liver. The highest concentrations of **ANGIOZYME(R)** were found in kidney and femur with all three routes. Because of the rapid and extensive absorption after extravascular injections, either i.p. or s.c. administration could be considered for use in pharmacodynamic studies examining the effects of **ANGIOZYME(R)** or other **ribozymes** with similar chemical modifications.

1999

2/3,AB/23 (Item 9 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
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12030188 BIOSIS NO.: 199900310707

Bioactivity of antiangiogenic ribozymes targeting **VEGF** receptor mRNA.
AUTHOR: Pavco Pamela(a); Cushman Cynthia; Sandberg Jennifer(a); Blatt Lawrence(a); Tressler Robert; Parry Tom(a)

AUTHOR ADDRESS: (a)Ribozyne Pharmaceuticals, Inc., Boulder, CO**USA

JOURNAL: FASEB Journal 13 (7):pA1320 April 23, 1999

CONFERENCE/MEETING: Annual Meeting of the American Societies for Experimental Biology on Biochemistry and Molecular Biology 99 San Francisco, California, USA May 16-20, 1999

SPONSOR: American Societies for Experimental Biology

ISSN: 0892-6638

RECORD TYPE: Citation

LANGUAGE: English

1999

2/3,AB/24 (Item 10 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
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11416102 BIOSIS NO.: 199800197434

Pharmacokinetics of a **VEGF** receptor targeted ribozymes (RPI.4610) in the mouse.

AUTHOR: Sandberg J A; Bouhana K S; Gallegos A M; Agrawal A; Grimm S; McOllough D; Parry T J

AUTHOR ADDRESS: Ribozyne Pharmaceuticals Inc., Boulder, CO 80301**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual

Meeting 39p524 March, 1998

CONFERENCE/MEETING: 89th Annual Meeting of the American Association for
Cancer Research New Orleans, Louisiana, USA March 28-April 1, 1998

SPONSOR: American Association for Cancer Research

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

1998

2/3,AB/25 (Item 11 from file: 5)

DIALOG(R) File 5:Biosis Previews(R)

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11413194 BIOSIS NO.: 199800194526

Antitumor and antimetastatic efficacy of a **ribozyme** targeting Fit-1
VEGF receptor mRNA.

AUTHOR: Parry T J(a); Bouhana K; Gallegos A M; Speirer K S; Agrawal A;
Pitot P; Tressler R J; Jensen K; Grimm S; McCollough D; Reynolds M A;
Pavco P

AUTHOR ADDRESS: (a)Ribozyme Pharm. Inc., Boulder, CO 80301**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual
Meeting 39p96 March, 1998

CONFERENCE/MEETING: 89th Annual Meeting of the American Association for
Cancer Research New Orleans, Louisiana, USA March 28-April 1, 1998

SPONSOR: American Association for Cancer Research

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

1998

2/3,AB/26 (Item 12 from file: 5)

DIALOG(R) File 5:Biosis Previews(R)

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10355733 BIOSIS NO.: 199698810651

Molecular modulation of vascular endothelial growth factor (VEGF)
expression in glioma cells by a **ribozyme**.

AUTHOR: Ke L C; Chen X S; Steck P A; Yung W K A

AUTHOR ADDRESS: Univ. Texas M.D. Anderson Cancer Cent., Houston, TX**USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual
Meeting 37 (0):p60 1996

CONFERENCE/MEETING: 87th Annual Meeting of the American Association for
Cancer Research Washington, D.C., USA April 20-24, 1996

ISSN: 0197-016X

RECORD TYPE: Citation

LANGUAGE: English

1996

=> s 120 and (deliver? (p) (ribozyme or antisense))

327194 DELIVER?
976 RIBOZYME
967 RIBOZYMES
1241 RIBOZYME
(RIBOZYME OR RIBOZYMES)
4886 ANTISENSE
1 ANTISENSES
4886 ANTISENSE
(ANTISENSE OR ANTISENSSES)
1070 DELIVER? (P) (RIBOZYME OR ANTISENSE)
L21 19 L20 AND (DELIVER? (P) (RIBOZYME OR ANTISENSE))

=> d ti,ab 1-19

US PAT NO: 5,840,710 [IMAGE AVAILABLE] L21: 1 of 19
TITLE: Cationic amphiphiles containing ester or ether-linked
lipophilic groups for intracellular **delivery** of
therapeutic molecules

ABSTRACT:

Novel cationic amphiphiles are provided that facilitate transport of biologically active (therapeutic) molecules into cells. The amphiphiles contain lipophilic groups derived from steroids, from mono or dialkylamines, or from alkyl or acyl groups; and cationic groups, protonatable at physiological pH, derived from amines, alkylamines or polyalkylamines. There are provided also therapeutic compositions prepared typically by contacting a dispersion of one or more cationic amphiphiles with the therapeutic molecules. Therapeutic molecules that can be **delivered** into cells according to the practice of the invention include DNA, **RNA**, and polypeptides. Representative uses of the therapeutic compositions of the invention include providing gene therapy, and **delivery** of **antisense** polynucleotides or biologically active polypeptides to cells. With respect to therapeutic compositions for gene therapy, the DNA is provided typically in the form of a plasmid for complexing with the cationic amphiphile. Novel and highly effective plasmid constructs are also disclosed, including those that are particularly effective at providing gene therapy for clinical conditions complicated by inflammation. Additionally, targeting of organs for gene therapy by intravenous administration of therapeutic compositions is described.

US PAT NO: 5,783,565 [IMAGE AVAILABLE] L21: 2 of 19
TITLE: Cationic amphiphiles containing spermine or spermidine
cationic group for intracellular **delivery** of
therapeutic molecules

ABSTRACT:

Novel cationic amphiphiles are provided that facilitate transport of biologically active (therapeutic) molecules into cells. The amphiphiles contain lipophilic groups derived from steroids, from mono or dialkylamines, or from alkyl or acyl groups; and cationic groups, protonatable at physiological pH, derived from amines, alkylamines or polyalkylamines. There are provided also therapeutic compositions prepared typically by contacting a dispersion of one or more cationic amphiphiles with the therapeutic molecules. Therapeutic molecules that can be **delivered** into cells according to the practice of the

- invention include DNA, RNA, and polypeptides. Representative uses of the therapeutic compositions of the invention include providing gene therapy, and **delivery** of **antisense** polynucleotides or biologically active polypeptides to cells. With respect to therapeutic compositions for gene therapy, the DNA is provided typically in the form of a plasmid for complexing with the cationic amphiphile. Novel and highly effective plasmid constructs are also disclosed, including those that are particularly effective at providing gene therapy for clinical conditions complicated by inflammation. Additionally, targeting of organs for gene therapy by intravenous administration of therapeutic compositions is described.

US PAT NO: 5,767,099 [IMAGE AVAILABLE] L21: 3 of 19
TITLE: Cationic amphiphiles containing amino acid or derivatized amino acid groups for intracellular **delivery** of therapeutic molecules

ABSTRACT:

Novel cationic amphiphiles are provided that facilitate transport of biologically active (therapeutic) molecules into cells. The amphiphiles contain lipophilic groups derived from steroids, from mono or dialkylamines, or from alkyl or acyl groups; and cationic groups, protonatable at physiological pH, derived from amines, alkylamines or polyalkylamines. There are provided also therapeutic compositions prepared typically by contacting a dispersion of one or more cationic amphiphiles with the therapeutic molecules. Therapeutic molecules that can be **delivered** into cells according to the practice of the invention include DNA, RNA, and polypeptides. Representative uses of the therapeutic compositions of the invention include providing gene therapy, and **delivery** of **antisense** polynucleotides or biologically active polypeptides to cells. With respect to therapeutic compositions for gene therapy, the DNA is provided typically in the form of a plasmid for complexing with the cationic amphiphile. Novel and highly effective plasmid constructs are also disclosed, including those that are particularly effective at providing gene therapy for clinical conditions complicated by inflammation. Additionally, targeting of organs for gene therapy by intravenous administration of therapeutic compositions is described.

US PAT NO: 5,767,073 [IMAGE AVAILABLE] L21: 4 of 19
TITLE: D4 gene and methods of use thereof

ABSTRACT:

The sequence, molecular structure and expression of a cDNA clone, denoted D4, of human and murine origin, preferentially expressed in hematopoietic cells is described herein. The human cDNA clone has been expressed in bacteria and the predicted 24 Kd protein purified. The protein has been used in studies of its biochemical function. As predicted on the basis of sequence, D4 can function as a GDP-dissociation inhibitor of at least several small GTP-binding proteins (CDC42 and rac). The D4 protein was used to generate a polyclonal antibody specific for the protein. The human cDNA was used to obtain several full length murine genomic clones. A clone has been analyzed and sequenced to use for the construction of a gene-targeting vector to produce animals deficient in D4 through disruption of the gene by homologous recombination. These animals can then be used as models for fundamental and applied research on the GTP-binding proteins.

US PAT NO: 5,747,471 [IMAGE AVAILABLE] L21: 5 of 19
TITLE: Cationic amphiphiles containing steroid lipophilic groups for intracellular **delivery** of therapeutic molecules

ABSTRACT:

Novel cationic amphiphiles are provided that facilitate transport of biologically active (therapeutic) molecules into cells. The amphiphiles

contain lipophilic groups derived from steroids, from mono or dialkylamines, or from alkyl or ester-linked alkyl groups; and cationic groups, protonatable at physiological pH, derived from amines, alkylamines or polyalkylamines. There are provided also therapeutic compositions prepared typically by contacting a dispersion of one or more cationic amphiphiles with the therapeutic molecules. Therapeutic molecules that can be delivered into cells according to the practice of the invention include DNA, RNA, and polypeptides. Representative uses of the therapeutic compositions of the invention include providing gene therapy, and delivery of antisense polynucleotides or biologically active polypeptides to cells. With respect to therapeutic compositions for gene therapy, the DNA is provided typically in the form of a plasmid for complexing with the cationic amphiphile. Novel and highly effective plasmid constructs are also disclosed, including those that are particularly effective at providing gene therapy for clinical conditions complicated by inflammation.

US PAT NO: 5,738,985 [IMAGE AVAILABLE] L21: 6 of 19
TITLE: Method for selective inactivation of viral replication

ABSTRACT:

Method for screening for an antiviral agent, by determining whether a potential agent interacts with a virus or cellular component which allows or prevents preferential translation of a virus RNA compared to a host RNA under virus infection conditions; and determining whether any interaction of the agent with the component reduces the level of translation of an RNA of the virus.

US PAT NO: 5,719,131 [IMAGE AVAILABLE] L21: 7 of 19
TITLE: Cationic amphiphiles containing dialkylamine lipophilic groups for intracellular delivery of therapeutic molecules

ABSTRACT:

Novel cationic amphiphiles are provided that facilitate transport of biologically active (therapeutic) molecules into cells. The amphiphiles contain lipophilic groups derived from steroids, from mono or dialkylamines, or from alkyl or acyl groups; and cationic groups, protonatable at physiological pH, derived from amines, alkylamines or polyalkylamines. There are provided also therapeutic compositions prepared typically by contacting a dispersion of one or more cationic amphiphiles with the therapeutic molecules. Therapeutic molecules that can be delivered into cells according to the practice of the invention include DNA, RNA, and polypeptides. Representative uses of the therapeutic compositions of the invention include providing gene therapy, and delivery of antisense polynucleotides or biologically active polypeptides to cells. With respect to therapeutic compositions for gene therapy, the DNA is provided typically in the form of a plasmid for complexing with the cationic amphiphile. Novel and highly effective plasmid constructs are also disclosed, including those that are particularly effective at providing gene therapy for clinical conditions complicated by inflammation. Additionally, targeting of organs for gene therapy by intravenous administration of therapeutic compositions is described.

US PAT NO: 5,705,385 [IMAGE AVAILABLE] L21: 8 of 19
TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

ABSTRACT:

Novel, hydrophobic lipid-nucleic acid complexes. The complexes are charge-neutralized and contain the nucleic acid in a non-condensed form. Manipulation of these complexes in either detergent-based or organic solvent-based systems leads to particle formation. Thus, the present

invention also provides methods of preparing lipid-nucleic acid particles which are useful for therapeutic delivery of nucleic acids. The particles are constructed via hydrophobic lipid-nucleic acid intermediates (or complexes). Upon removal of a solubilizing component (i.e., detergent or an organic solvent) the nucleic acid forms a particle with lipids and is protected from degradation. The particles thus formed are suitable for use in intravenous nucleic acid transfer as they are stable in circulation, of a size required for pharmacodynamic behavior resulting in access to extravascular sites and target cell populations.

US PAT NO: 5,674,911 [IMAGE AVAILABLE] L21: 9 of 19
TITLE: Antiinfective polyoxypropylene/polyoxyethylene copolymers and methods of use

ABSTRACT:

The present invention comprises novel preparations of polyoxypropylene/polyoxyethylene copolymers which retain the therapeutic activity of the commercial preparations, but substantially reduce the undesirable effects which are inherent in the prior art preparations. Because the preparations of polyoxypropylene/polyoxyethylene copolymers which comprise the present invention are a less polydisperse population of molecules than the prior art polyoxypropylene/polyoxyethylene copolymers, the biological activity of the copolymers is better defined and more predictable and the cardiotoxicity inherent in the native copolymers is substantially reduced.

US PAT NO: 5,627,270 [IMAGE AVAILABLE] L21: 10 of 19
TITLE: Glycosylated steroid derivatives for transport across biological membranes and process for making and using same

ABSTRACT:

Novel glycosylated steroid derivatives for facilitating the transport of compounds across biological membranes, either in admixture or as conjugates, are disclosed. A novel process for efficient synthesis of these glycosylated steroid derivatives, using activated glycosyl sulfoxide intermediates is provided. Methods for the permeabilization of membranes and the enhancement of the activity of predetermined compounds are also provided.

US PAT NO: 5,622,712 [IMAGE AVAILABLE] L21: 11 of 19
TITLE: N-[.omega., (.omega.-1)-dialkyloxy]- and N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N, N, N-tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴, R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 5,585,478 [IMAGE AVAILABLE] L21: 12 of 19
TITLE: D4 gene and methods of use thereof

ABSTRACT:

The sequence, molecular structure and expression of a cDNA clone, denoted D4, of human and murine origin, preferentially expressed in hematopoietic cells is described herein. The human cDNA clone has been expressed in bacteria and the predicted 24 Kd protein purified. The protein has been used in studies of its biochemical function. As predicted on the basis of sequence, D4 can function as a GDP-dissociation inhibitor of at least

several small GTP-binding proteins (CDC42 and rac). The D4 protein was used to generate a polyclonal antibody specific for the protein. The human cDNA was used to obtain several full length murine genomic clones. A clone has been analyzed and sequenced to use for the construction of a gene-targeting vector to produce animals deficient in D4 through disruption of the gene by homologous recombination. These animals can then be used as models for fundamental and applied research on the GTP-binding proteins.

US PAT NO: 5,550,289 [IMAGE AVAILABLE] L21: 13 of 19
TITLE: N-(1, (1-1)-dialkyloxy)-and N-(1, (1-1)-dialkenyloxy alk-1-yl-N,N,N-tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 5,545,412 [IMAGE AVAILABLE] L21: 14 of 19
TITLE: N-[1, (1-1)-dialkyloxy]-and N-[1, (1-1)-dialkenyloxy]-alk-1-yl-n,n,n-tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino, n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 5,366,737 [IMAGE AVAILABLE] L21: 15 of 19
TITLE: N-[.omega., (.omega.-1)-dialkyloxy]- and N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N,N,N,- tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 5,208,036 [IMAGE AVAILABLE] L21: 16 of 19
TITLE: N-[.omega., (.omega.-1)-dialkyloxy]- and N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N,N,N,- tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of

R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 5,049,386 [IMAGE AVAILABLE] L21: 17 of 19
TITLE: N-.(omega.,(omega.-1)-dialkyloxy)- and
N-(.omega.,(.omega.-1)-dialkenyloxy)Alk-1-YL-N,N,N-
tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 4,946,787 [IMAGE AVAILABLE] L21: 18 of 19
TITLE: N-(.omega.,(omega.-1)-dialkyloxy)- and
N-(.omega.,(.omega.-1)-dialkenyloxy)-alk-1-yl-N,N,N-
tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

US PAT NO: 4,897,355 [IMAGE AVAILABLE] L21: 19 of 19
TITLE: N[.omega.,(omega.-1)-dialkyloxy]- and
N-[.omega.,(.omega.-1)-dialkenyloxy]-alk-1-yl-N,N,N-
tetrasubstituted ammonium **lipids** and uses therefor

ABSTRACT:

This invention relates to compounds of the formula ##STR1## or an optical isomer thereof wherein R.¹ and R.² are the same or different and are an alkyl or alkenyl group of 6 to 24 carbon atoms; R.³, R.⁴ and R.⁵ are the same or different and are alkyl of 1 to 8 carbon atoms, aryl, aralkyl of 7 to 11 carbon atoms, or when two or three of R.³, R.⁴, and R.⁵ are taken together to form quinuclidino, piperidino, pyrrolidino, or morpholino; n is 1 to 8; and X is a pharmaceutically acceptable anion.

=> s (vascular endothelial growth factor receptor) or vegfr

20784 VASCULAR
11 VASCULARS
20785 VASCULAR
(VASCULAR OR VASCULARS)
6491 ENDOTHELIAL
3 ENDOTHELIALS
6491 ENDOTHELIAL
(ENDOTHELIAL OR ENDOTHELIALS)
149473 GROWTH
2659 GROWTHS
150210 GROWTH
(GROWTH OR GROWTHS)
267093 FACTOR
240363 FACTORS
419084 FACTOR
(FACTOR OR FACTORS)
32115 RECEPTOR
21408 RECEPTORS
38035 RECEPTOR
(RECEPTOR OR RECEPTORS)
25 VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR
(VASCULAR (W) ENDOTHELIAL (W) GROWTH (W) FACTOR (W) RECEPTOR)
1 VEGFR
1 VEGFRS
1 VEGFR
(VEGFR OR VEGFRS)
L22 26 (VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR) OR VEGFR

=> s l22 and (antisense or ribozyme)

4886 ANTISENSE
1 ANTISENSES
4886 ANTISENSE
(ANTISENSE OR ANTISENSSES)
976 RIBOZYME
967 RIBOZYMES
1241 RIBOZYME
(RIBOZYME OR RIBOZYMES)
L23 2 L22 AND (ANTISENSE OR RIBOZYME)

=> d ti,ab 1-2

US PAT NO: 5,837,283 [IMAGE AVAILABLE] L23: 1 of 2
TITLE: Cationic lipid compositions targeting angiogenic
endothelial cells

ABSTRACT:

Angiogenic endothelial cells are selectively targeted with lipid/DNA complexes or cationic liposomes containing a substance which affects the targeted cells by inhibiting or promoting their growth. A site of angiogenesis can be precisely located by administering cationic liposomes containing a detectable label. The complexes may comprise nucleotide constructs which are comprised of promoters which are selectively and exclusively activated in the environment of an angiogenic endothelial cell.

US PAT NO: 5,776,7 IMAGE AVAILABLE]
TITLE: FLT4, a receptor tyrosine kinase

2 of 2

ABSTRACT:

FLT4 gene, expression systems and proteins are provided for use in diagnosis and treatment of conditions related to the tyrosine kinase receptor encoded by the FLT4 gene.

3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09597121 98334670

Transcription factors Sp1 and Sp3 alter **vascular endothelial growth factor receptor** expression through a novel recognition sequence.

Hata Y; Duh E; Zhang K; Robinson GS; Aiello LP
Research Division, Joslin Diabetes Center, Boston, Massachusetts 02215,
USA.

J Biol Chem (UNITED STATES) Jul 24 1998, 273 (30) p19294-303, ISSN
0021-9258 Journal Code: HIV

Contract/Grant No.: EY-10827, EY, NEI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Kinase domain receptor (KDR) is a high affinity, endothelial cell-specific, autophosphorylating tyrosine kinase receptor for vascular endothelial growth factor. This transcriptionally regulated receptor is a critical mediator of endothelial cell (EC) growth and vascular development. In this study, we identify a DNA element modulating KDR promoter activity and evaluate the nuclear binding proteins accounting for a portion of the cell-type specificity of the region. KDR promoter luciferase activity was retained within -85/+296 and was 10-30-fold higher in EC than non-EC. Electrophoretic mobility shift assays demonstrated specific nuclear protein binding to -85/-64, and single point mutations suggested important binding nucleotides between -79/-68 with five critical bases between -74/-70 (5'-CTCCT-3'). DNA-protein complexes were displaced by Sp1 consensus sequence oligodeoxynucleotides and supershifted by Sp1- and Sp3-specific antibodies. Sp1 and Sp3 protein in EC nuclear extracts bound the -79/-68 region even when all surrounding classic Sp1 recognition sites were removed. Sp1 protein in nuclear extracts was 4-24-fold higher in EC than non-EC, whereas Sp3 was 3-7-fold higher. Sp1/Sp3 ratios in EC were 2-10-fold higher. Overexpression of Sp1 protein increased KDR promoter activity 3-fold in both EC and non-EC, whereas simultaneous co-expression of Sp3 attenuated this response. An Sp1 consensus sequence cis element "decoy" reduced EC KDR promoter activity and mRNA expression by 85 and 69%, respectively. An **antisense** phosphorothioate oligodeoxynucleotide to Sp1 inhibited Sp1 and KDR protein expression by 66 and 68%, respectively, without changing Sp3 protein expression. These data illustrate that Sp1 and Sp3 modulate KDR promoter activity through a novel recognition binding sequence. However, since Sp1-mediated promoter activation is attenuated by Sp3, endothelial selective KDR promoter activity may be partially regulated by variations in the Sp1/Sp3 ratio.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08083114 95101869

Vascular endothelial growth factor receptor
localization and activation in human trophoblast and choriocarcinoma cells.
Charnock-Jones DS; Sharkey AM; Boocock CA; Ahmed A; Plevin R; Ferrara N;
Smith SK

Department of Obstetrics and Gynaecology, University of Cambridge, Rosie Maternity Hospital, England.

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Vascular endothelial growth factor (VEGF; also known as vascular permeability factor) is a secreted angiogenic growth factor. It is highly specific for endothelial cells, and its receptor, the fms-like tyrosine kinase (flt), has been localized only to endothelial cells in vivo. Here we describe the expression of mRNA encoding flt in human trophoblast as revealed by *in situ* hybridization. This mRNA is highly expressed in the cytotrophoblast shell and columns and also highly expressed by the extravillous trophoblast (EVT) in the maternal decidua both in the first trimester and at term. The trophoblast-like choriocarcinoma cell line BeWo also expresses this receptor and the related receptor, kinase domain-containing receptor (KDR), which is also a receptor for VEGF. Treatment of the cell line BeWo with VEGF165 stimulated ³H-thymidine incorporation and tyrosine phosphorylation of MAP (mitogen-activated protein) kinase in a time- and dose-dependent fashion. This study is the first demonstration of the presence of flt on non-endothelial cells in vivo and suggests a role for VEGF in the growth and differentiation of cytotrophoblast at implantation.

3/3,AB/3 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11606080 BIOSIS NO.: 199800387815
Down-regulation of platelet endothelial cell adhesion molecule-1 results in thrombospondin-1 expression and concerted regulation of endothelial cell phenotype.

AUTHOR: Sheibani Nader(a); Frazier William A
AUTHOR ADDRESS: (a)Dep. Biochem. and Molecular Biophysics, Washington Univ.
Sch. Med., 660 South Euclid Ave., Box 8, USA

JOURNAL: Molecular Biology of the Cell 9 (4):p701-713 April, 1998

ISSN: 1059-1524

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: bEND.3 cells are polyoma middle T-transformed mouse brain endothelial cells that express very little or no thrombospondin-1, a natural inhibitor of angiogenesis, but express high levels of platelet endothelial cell adhesion molecule-1 (PECAM-1) that localizes to sites of cell-cell contact. Here, we have examined the role of PECAM-1 in regulation of bEND.3 cell proliferation, migration, morphogenesis, and hemangioma formation. We show that down-regulating PECAM-1 expression by **antisense** transfection of bEND.3 cells has a dramatic effect on their morphology, proliferation, and morphogenesis on Matrigel. There is an optimal level for PECAM-1 expression such that high levels of PECAM-1 inhibit, whereas moderate levels of PECAM-1 stimulate, endothelial cell morphogenesis. The down-regulation of PECAM-1 in bEND.3 cells resulted in reexpression of endogenous thrombospondin-1 and its antiangiogenic receptor CD36. The expression of the vascular endothelial growth factor receptors flk-1 and flt-1, as well as integrins and metalloproteinases (which are involved in angiogenesis), were also affected. These observations are consistent with the changes observed in proliferation, migration, and adhesion characteristics of the **antisense**-transfected bEND.3 cells as well as with their lack of ability to form hemangiomas in mice. Thus, a reciprocal relationship exists between thrombospondin-1 and PECAM-1 expression, such that these two molecules appear to be constituents of a "switch" that regulates in concert many components of the angiogenic and differentiated phenotypes of endothelial

cells.

3/3,AB/4 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11416102 BIOSIS NO.: 199800197434
Pharmacokinetics of a VEGF receptor targeted ribozymes (RPI.4610) in the mouse.

AUTHOR: Sandberg J A; Bouhana K S; Gallegos A M; Agrawal A; Grimm S;
McCollough D; Parry T J
AUTHOR ADDRESS: Ribozyme Pharmaceuticals Inc., Boulder, CO 80301, USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 39p524 March, 1998

CONFERENCE/MEETING: 89th Annual Meeting of the American Association for Cancer Research New Orleans, Louisiana, USA March 28-April 1, 1998
SPONSOR: American Association for Cancer Research

ISSN: 0197-016X
RECORD TYPE: Citation
LANGUAGE: English

3/3,AB/5 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11413194 BIOSIS NO.: 199800194526
Antitumor and antimetastatic efficacy of a **ribozyme** targeting Fit-1 VEGF receptor mRNA.

AUTHOR: Parry T J(a); Bouhana K; Gallegos A M; Speirer K S; Agrawal A;
Pitot P; Tressler R J; Jensen K; Grimm S; McCollough D; Reynolds M A;
Pavco P
AUTHOR ADDRESS: (a)Ribozyme Pharm. Inc., Boulder, CO 80301, USA

JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 39p96 March, 1998

CONFERENCE/MEETING: 89th Annual Meeting of the American Association for Cancer Research New Orleans, Louisiana, USA March 28-April 1, 1998
SPONSOR: American Association for Cancer Research

ISSN: 0197-016X
RECORD TYPE: Citation
LANGUAGE: English

3/3,AB/6 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10995262 BIOSIS NO.: 199799616407
Cell-surface perturbations of the epidermal growth factor and vascular endothelial growth factor receptors by phosphorothioate oligodeoxynucleotides.

AUTHOR: Rockwell Patricia; O'Connor William J; King Karen; Goldstein Neil I ; Zhang L M; Stein C A(a)
AUTHOR ADDRESS: (a)Dep. Med., Columbia Univ., New York, NY 10032, USA

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 94 (47):p6523-6528 1997

ISSN: 0027-8424

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: **Antisense** oligodeoxynucleotides offer potential as therapeutic agents to inhibit gene expression. Recent evidence indicates that oligodeoxynucleotides designed to target specific nucleic acid sequences can interact nonspecifically with proteins. This report describes the interactive capabilities of phosphorothioate oligodeoxynucleotides of defined sequence and length with two essential protein tyrosine receptors, flk-1 and epidermal growth factor receptor (EGFR), and their effects on receptor signaling in a transfected and tumor cell line, respectively. Phosphorothioate oligodeoxynucleotides bound to the cell surface, as demonstrated by fluorescence-activated cell-sorter analyses (FACS), and perturbed receptor activation in the presence and absence of cognate ligands, EGF (EGFR) and vascular endothelial growth factor (flk-1), in phosphorylation assays. Certain phosphorothioate oligodeoxynucleotides interacted relatively selectively with flk-1 and partially blocked the binding of specific anti-receptor monoclonal antibodies to target sites. They stimulated EGFR phosphorylation in the absence of EGF but antagonized ligand-mediated activation of EGFR and flk-1. In vivo studies showed that a nonspecific phosphorothioate oligodeoxynucleotide suppressed the growth of glioblastoma in a mouse model of tumorigenesis. These results emphasize the capacity of phosphorothioate oligodeoxynucleotides to interact with cells in a sequence-selective non-**antisense** manner, while associating with cellular membrane proteins in ways that can inhibit cellular metabolic activities.

3/3, AB/7 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10149643 BIOSIS NO.: 199698604561
Possible participation of autocrine and paracrine vascular endothelial growth factors in hypoxia-induced proliferation of endothelial cells and pericytes.

AUTHOR: Nomura Motohiro; Yamagishi Sho-Ichi; Harada Shin-Ichi; Hayashi Yasuhiko; Yamashima Tetsumori; Yamashita Junkoh; Yamamoto Hiroshi(a)

AUTHOR ADDRESS: (a)Dep. Biochem., Kanazawa Univ. Sch. Med., 13-1 Takara-machi, Kanazawa 920, Japan

JOURNAL: Journal of Biological Chemistry 270 (47):p28316-28324 1995

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Hypoxia is the principal factor that causes angiogenesis. These experiments were conducted to explore how it induces the proliferation of vascular cells, a key step in angiogenesis. Human umbilical vein endothelial cells and bovine retinal pericytes were grown in controlled atmosphere culture chambers containing various concentrations of oxygen. The numbers of both endothelial cells and pericytes increased significantly under hypoxic conditions; the O₂ concentrations that achieved maximal growth promotion were 10% for endothelial cells and 2.5% for pericytes. Quantitative reverse transcription-polymerase chain reaction analysis revealed that mRNAs coding for the secretory forms of vascular endothelial growth factor (VEGF), a mitogen specific to endothelial cells, were present in both endothelial cells and pericytes and that their levels increased significantly in the two cell types as

the atmospheric O₂ concentration decreased. The two genes for VEGF receptors, kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase 1 (flt1), were found to be constitutively expressed in endothelial cells, and their relative mRNA levels were ranked in that order. On the other hand, only flt1 mRNA was detected in pericytes under hypoxic conditions. Furthermore, most **antisense** oligodeoxyribonucleotides complementary to VEGF mRNAs efficiently inhibited DNA synthesis in endothelial cells cultured under hypoxic conditions. These results indicate that autocrine and paracrine VEGFs may take part in the hypoxia-induced proliferation of endothelial cells.

=> s 12 and phosphatidyl choline

2343 PHOSPHATIDYL
7382 CHOLINE
412 CHOLINES
7636 CHOLINE
(CHOLINE OR CHOLINES)
1477 PHOSPHATIDYL CHOLINE
(PHOSPHATIDYL(W) CHOLINE)
L3 2 L2 AND PHOSPHATIDYL CHOLINE

=> d ti 1-2

US PAT NO: 5,846,517 [IMAGE AVAILABLE] L3: 1 of 2
TITLE: Methods for diagnostic imaging using a renal contrast agent and a vasodilator

US PAT NO: 5,817,856 [IMAGE AVAILABLE] L3: 2 of 2
TITLE: Radiation-protective phospholipid and method

=> s 12 and cholesterol

13190 CHOLESTEROL
276 CHOLESTEROLS
13261 CHOLESTEROL
(CHOLESTEROL OR CHOLESTEROLS)
L4 27 L2 AND CHOLESTEROL

5,820873

=> s 12 not 14

L5 6 L2 NOT L4

=> d ti 1-6

US PAT NO: 5,885,557 [IMAGE AVAILABLE] L5: 1 of 6
TITLE: Compositions useful in the phototherapeutic treatment of proliferative skin disorders

US PAT NO: 5,756,108 [IMAGE AVAILABLE] L5: 2 of 6
TITLE: Oily phase in an aqueous phase dispersion stabilized by cubic gel particles and method of making

US PAT NO: 5,705,145 [IMAGE AVAILABLE] L5: 3 of 6
TITLE: Skin tanning compositions and method

US PAT NO: 5,490,980 [IMAGE AVAILABLE] L5: 4 of 6
TITLE: Covalent bonding of active agents to skin, hair or nails

US PAT NO: 5,411,734 [IMAGE AVAILABLE] L5: 5 of 6
TITLE: Non-irritating .alpha.-hydroxy carboxylic acid compositions

US PAT NO: 5,368,857 [IMAGE AVAILABLE] L5: 6 of 6
TITLE: Ceramide cosmetic compositions

=> s dodac

L6

10 DODAC

=> s 16 and liposome

4650 LIPOSOME
7743 LIPOSOMES
8935 LIPOSOME
(LIPOSOME OR LIPOSOMES)

L7 7 L6 AND LIPOSOME

=> d ti 1-7

US PAT NO: 5,885,613 [IMAGE AVAILABLE] L7: 1 of 7
TITLE: Bilayer stabilizing components and their use in forming
programmable fusogenic **liposomes**

US PAT NO: 5,843,901 [IMAGE AVAILABLE] L7: 2 of 7
TITLE: LHRH antagonist peptides

US PAT NO: 5,820,873 [IMAGE AVAILABLE] L7: 3 of 7
TITLE: Polyethylene glycol modified ceramide lipids and
liposome uses thereof

US PAT NO: 5,785,992 [IMAGE AVAILABLE] L7: 4 of 7
TITLE: Compositions for the introduction of polyanionic materials
into cells

US PAT NO: 5,780,435 [IMAGE AVAILABLE] L7: 5 of 7
TITLE: Methods for treating prostate cancer with LHRH-R
antagonists

US PAT NO: 5,753,613 [IMAGE AVAILABLE] L7: 6 of 7
TITLE: Compositions for the introduction of polyanionic materials
into cells

US PAT NO: 5,705,385 [IMAGE AVAILABLE] L7: 7 of 7
TITLE: Lipid-nucleic acid particles prepared via a hydrophobic
lipid-nucleic acid complex intermediate and use for gene
transfer

=> s dotap

L8 156 DOTAP

=> s 18 and liposome

4650 LIPOSOME
7743 LIPOSOMES
8935 LIPOSOME
(LIPOSOME OR LIPOSOMES)

L9 122 L8 AND LIPOSOME

=> s 18 and lipid

17228 LIPID
12861 LIPIDS
22925 LIPID
(LIPID OR LIPIDS)

L10 128 L8 AND LIPID

=> s 110 and peg

23020 PEG

10015 PEGS
28407 PEG
(PEG OR PEGS)
L11 29 L10 AND PEG

=> s l11 and ceramide

560 CERAMIDE
417 CERAMIDES
786 CERAMIDE
(CERAMIDE OR CERAMIDES)
L12 8 L11 AND CERAMIDE

=> s peg (2a) ceramide

23020 PEG
10015 PEGS
28407 PEG
(PEG OR PEGS)
560 CERAMIDE
417 CERAMIDES
786 CERAMIDE
(CERAMIDE OR CERAMIDES)
L13 4 PEG (2A) CERAMIDE

=> d ti 1-4

| | | |
|------------|---|-------------|
| US PAT NO: | 5,885,613 [IMAGE AVAILABLE] | L13: 1 of 4 |
| TITLE: | Bilayer stabilizing components and their use in forming programmable fusogenic liposomes | |
| US PAT NO: | 5,837,282 [IMAGE AVAILABLE] | L13: 2 of 4 |
| TITLE: | Ionophore-mediated liposome loading | |
| US PAT NO: | 5,820,873 [IMAGE AVAILABLE] | L13: 3 of 4 |
| TITLE: | Polyethylene glycol modified ceramide lipids and liposome uses thereof | |
| US PAT NO: | 5,705,385 [IMAGE AVAILABLE] | L13: 4 of 4 |
| TITLE: | Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer | |

=> d 112 -18 ti

8 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET
SIZE
ENTER ANSWER NUMBER OR RANGE (1):

ENTER ANSWER NUMBER OR RANGE (1):

US PAT NO: 5,885,613 [IMAGE AVAILABLE] L12: 1 of 8
TITLE: Bilayer stabilizing components and their use in forming
programmable fusogenic liposomes

=> d 112 1-8 ti

US PAT NO: 5,885,613 [IMAGE AVAILABLE] L12: 1 of 8
TITLE: Bilayer stabilizing components and their use in forming
programmable fusogenic liposomes

US PAT NO: 5,874,062 [IMAGE AVAILABLE] L12: 2 of 8

TITLE: Methods of computed tomography using perfluorocarbon
gaseous filled microspheres as contrast agents

US PAT NO: 5,846,517 [IMAGE AVAILABLE] L12: 3 of 8
TITLE: Methods for diagnostic imaging using a renal contrast
agent and a vasodilator

US PAT NO: 5,837,221 [IMAGE AVAILABLE] L12: 4 of 8
TITLE: Polymer-lipid microencapsulated gases for use as
imaging agents

US PAT NO: 5,785,992 [IMAGE AVAILABLE] L12: 5 of 8
TITLE: Compositions for the introduction of polyanionic materials
into cells

US PAT NO: 5,736,121 [IMAGE AVAILABLE] L12: 6 of 8
TITLE: Stabilized homogenous suspensions as computed tomography
contrast agents

US PAT NO: 5,733,572 [IMAGE AVAILABLE] L12: 7 of 8
TITLE: Gas and gaseous precursor filled microspheres as topical
and subcutaneous delivery vehicles

US PAT NO: 5,705,385 [IMAGE AVAILABLE] L12: 8 of 8
TITLE: Lipid-nucleic acid particles prepared via a
hydrophobic lipid-nucleic acid complex intermediate
and use for gene transfer

=> d his

(FILE 'USPAT' ENTERED AT 08:06:53 ON 03 MAY 1999)

L1 34 S PEG (P) CERAMIDE
L2 33 S L1 AND LIPID
L3 2 S L2 AND PHOSPHATIDYL CHOLINE
L4 27 S L2 AND CHOLESTEROL
L5 6 S L2 NOT L4
L6 10 S DODAC
L7 7 S L6 AND LIPOSOME
L8 156 S DOTAP
L9 122 S L8 AND LIPOSOME
L10 128 S L8 AND LIPID
L11 29 S L10 AND PEG
L12 8 S L11 AND CERAMIDE
L13 4 S PEG (2A) CERAMIDE

Peg(β) ceramide

| | | |
|------------|---|--------------|
| US PAT NO: | 5,885,613 [IMAGE AVAILABLE] | L2: 1 of 33 |
| TITLE: | Bilayer stabilizing components and their use in forming programmable fusogenic liposomes | |
| US PAT NO: | 5,885,557 [IMAGE AVAILABLE] | L2: 2 of 33 |
| TITLE: | Compositions useful in the phototherapeutic treatment of proliferative skin disorders | |
| US PAT NO: | 5,855,893 [IMAGE AVAILABLE] | L2: 3 of 33 |
| TITLE: | Trichodesma lanicum seed extract as an anti-irritant in compositions containing hydroxy acids or retinoids | |
| US PAT NO: | 5,846,517 [IMAGE AVAILABLE] | L2: 4 of 33 |
| TITLE: | Methods for diagnostic imaging using a renal contrast agent and a vasodilator | |
| US PAT NO: | 5,837,282 [IMAGE AVAILABLE] | L2: 5 of 33 |
| TITLE: | Ionophore-mediated liposome loading | |
| US PAT NO: | 5,820,873 [IMAGE AVAILABLE] | L2: 6 of 33 |
| TITLE: | Polyethylene glycol modified ceramide lipids and liposome uses thereof | |
| US PAT NO: | 5,817,856 [IMAGE AVAILABLE] | L2: 7 of 33 |
| TITLE: | Radiation-protective phospholipid and method | |
| US PAT NO: | 5,800,833 [IMAGE AVAILABLE] | L2: 8 of 33 |
| TITLE: | Method for loading lipid vesicles | |
| US PAT NO: | 5,785,992 [IMAGE AVAILABLE] | L2: 9 of 33 |
| TITLE: | Compositions for the introduction of polyanionic materials into cells | |
| US PAT NO: | 5,785,987 [IMAGE AVAILABLE] | L2: 10 of 33 |
| TITLE: | Method for loading lipid vesicles | |
| US PAT NO: | 5,766,628 [IMAGE AVAILABLE] | L2: 11 of 33 |
| TITLE: | Bath and shower composition having vesicle-forming properties and method for the production and use thereof | |
| US PAT NO: | 5,756,108 [IMAGE AVAILABLE] | L2: 12 of 33 |
| TITLE: | Oily phase in an aqueous phase dispersion stabilized by cubic gel particles and method of making | |
| US PAT NO: | 5,736,121 [IMAGE AVAILABLE] | L2: 13 of 33 |
| TITLE: | Stabilized homogenous suspensions as computed tomography contrast agents | |
| US PAT NO: | 5,705,385 [IMAGE AVAILABLE] | L2: 14 of 33 |
| TITLE: | Lipid -nucleic acid particles prepared via a hydrophobic lipid -nucleic acid complex intermediate and use for gene transfer | |
| US PAT NO: | 5,705,145 [IMAGE AVAILABLE] | L2: 15 of 33 |
| TITLE: | Skin tanning compositions and method | |
| US PAT NO: | 5,690,947 [IMAGE AVAILABLE] | L2: 16 of 33 |
| TITLE: | Borage seed oil as an anti-irritant in compositions | |

containing hydroxy acids or retinoids

| | | |
|------------|---|--------------|
| US PAT NO: | 5,662,936 [IMAGE AVAILABLE] | L2: 17 of 33 |
| TITLE: | Reduction of liposome-induced adverse physiological reactions | |
| US PAT NO: | 5,627,056 [IMAGE AVAILABLE] | L2: 18 of 33 |
| TITLE: | Method of synthesizing lipids and cosmetic composition comprising them | |
| US PAT NO: | 5,614,214 [IMAGE AVAILABLE] | L2: 19 of 33 |
| TITLE: | Reduction of liposome-induced adverse physiological reactions | |
| US PAT NO: | 5,578,641 [IMAGE AVAILABLE] | L2: 20 of 33 |
| TITLE: | Cosmetic composition | |
| US PAT NO: | 5,554,366 [IMAGE AVAILABLE] | L2: 21 of 33 |
| TITLE: | Skin care method and composition | |
| US PAT NO: | 5,490,980 [IMAGE AVAILABLE] | L2: 22 of 33 |
| TITLE: | Covalent bonding of active agents to skin, hair or nails | |
| US PAT NO: | 5,476,671 [IMAGE AVAILABLE] | L2: 23 of 33 |
| TITLE: | Synthetic ceramides and their use in cosmetic compositions | |
| US PAT NO: | 5,476,661 [IMAGE AVAILABLE] | L2: 24 of 33 |
| TITLE: | Compositions for topical application to skin, hair and nails | |
| US PAT NO: | 5,439,935 [IMAGE AVAILABLE] | L2: 25 of 33 |
| TITLE: | Skin care method and composition | |
| US PAT NO: | 5,415,855 [IMAGE AVAILABLE] | L2: 26 of 33 |
| TITLE: | Cosmetic composition | |
| US PAT NO: | 5,411,734 [IMAGE AVAILABLE] | L2: 27 of 33 |
| TITLE: | Non-irritating .alpha.-hydroxy carboxylic acid compositions | |
| US PAT NO: | 5,401,517 [IMAGE AVAILABLE] | L2: 28 of 33 |
| TITLE: | Cosmetic method for treatment of skin | |
| US PAT NO: | 5,382,432 [IMAGE AVAILABLE] | L2: 29 of 33 |
| TITLE: | Cosmetic method for treatment of skin | |
| US PAT NO: | 5,368,857 [IMAGE AVAILABLE] | L2: 30 of 33 |
| TITLE: | Ceramide cosmetic compositions | |
| US PAT NO: | 5,326,565 [IMAGE AVAILABLE] | L2: 31 of 33 |
| TITLE: | Cosmetic composition | |
| US PAT NO: | 5,206,020 [IMAGE AVAILABLE] | L2: 32 of 33 |
| TITLE: | Synthetic pseudoceramide and cosmetic compositions thereof | |
| US PAT NO: | 5,198,210 [IMAGE AVAILABLE] | L2: 33 of 33 |
| TITLE: | Cosmetic composition | |

mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis.

Porteous DJ; Dorin JR; McLachlan G; Davidson-Smith H; Davidson H; Stevenson BJ; Carothers AD; Wallace WA; Moralee S; Hoenes C; Kallmeyer G; Michaelis U; Naujoks K; Ho LP; Samways JM; Imrie M; Greening AP; Innes JA
MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK.

Gene Ther (ENGLAND) Mar 1997, 4 (3) p210-8, ISSN 0969-7128

Journal Code: CCE

Languages: ENGLISH

Document type: CLINICAL TRIAL; JOURNAL ARTICLE; RANDOMIZED CONTROLLED TRIAL

In cystic fibrosis (CF), mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene results in defective transepithelial ion transport, leading to life shortening inflammatory lung disease. Before lung studies, we tested the safety and efficacy of gene delivery to the nasal epithelium of CF patients using pCMV-CFTR-DOTAP cationic liposome complex. A single dose of 400 micrograms pCMV-CFTR:2.4 mg DOTAP was administered in a randomised, double-blinded fashion to the nasal epithelium of eight CF patients, with a further eight receiving buffer only. Patients were monitored for signs and symptoms for 2 weeks before treatment and 4 weeks after treatment. Inflammatory cells were quantified in a nasal biopsy taken 3 days after treatment. There was no evidence for excess nasal inflammation, circulating inflammatory markers or other adverse events ascribable to active treatment. Gene transfer and expression were assayed by the polymerase chain reaction. Transgene DNA was detected in seven of the eight treated patients up to 28 days after treatment and vector derived CFTR mRNA in two of the seven patients at +3 and +7 days. Transepithelial ion transport was assayed before and after treatment by nasal potential difference during drug perfusion and by SPQ fluorescence halide ion conductance. Partial, sustained correction of CFTR-related functional changes toward normal values were detected in two treated patients. The level of gene transfer and functional correction were comparable to those reported previously using adenoviral vectors or another DNA-liposome complex, but here were sustained and uncompromised by false positives. These results justify further studies with pCMV-CFTR-DOTAP aimed at treating CF lung disease.

18/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155: MEDLINE(R)
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09033339 97207025

Pulmonary surfactant inhibits cationic liposome-mediated gene delivery to respiratory epithelial cells in vitro.

Duncan JE; Whitsett JA; Horowitz AD

Duke University School of Medicine, Durham, NC 27710, USA.

Hum Gene Ther (UNITED STATES) Mar 1 1997, 8 (4) p431-8, ISSN 1043-0342 Journal Code: A12

Contract/Grant No.: HL51832, HL, NHLBI; HL54929, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Cationic lipid-mediated transfection of the alveolar epithelium in vivo will require exposure of plasmid DNA and cationic lipids to endogenous surfactant lipids and proteins in the alveolar space. Effects of pulmonary surfactant and of surfactant constituents on transfection in vitro of two respiratory epithelial cell lines (MLE-15 and H441) with a plasmid encoding the luciferase reporter gene were studied using two cationic lipid formulations: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide/cholesterol (DMRIE/C) and 1,2-dioleoyl-3-trimethylammonium propane/dioleoyl phosphatidylethanolamine (DOTAP /DOPE). Gene expression, as assessed by luciferase activity, decreased as increasing concentrations of natural surfactant were added to cationic lipid-DNA complexes. Incorporation of phospholipids DOPC/DOPG or surfactant proteins SP-B or SP-C in the cationic lipid formulation inhibited transfection. A

fluorescent lipid mixing assay was used to determine the effects of surfactant proteins SP-B and SP-C on mixing between cationic lipid-DNA complexes and surfactant lipid vesicles. Mixing between DOPC/DOPG vesicles and cationic lipid-DNA complexes in the absence of added proteins amounted to 10-20%. Addition of SP-B or SP-C increased the mixing of DOPC/DOPG vesicles with DOTAP/DOPE-DNA complexes, but not DMRIEC-DNA complexes. These results demonstrate that pulmonary surfactant lipids and proteins inhibit transfection with cationic lipid-DNA complexes *in vitro*, and may therefore represent a barrier to gene transfer in the lung.

18/3,AB/12 (Item 12 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08886556 97001211

Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene **delivery**.

Plank C; Mechtler K; Szoka FC Jr; Wagner E
University of California, School of Pharmacy, San Francisco 94143-0446,
USA.

Hum Gene Ther (UNITED STATES) Aug 1 1996, 7 (12) p1437-46, ISSN 1043-0342 Journal Code: A12

Contract/Grant No.: DK46052, DK, NIDDK; P30 DK47766, DK, NIDDK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have examined the complement-activating properties of synthetic cationic molecules and their complexes with DNA. Commonly used gene **delivery** vehicles include complexes of DNA with polylysine of various chain lengths, transferrin-polylysine, a fifth-generation poly(amidoamine) (PAMAM) dendrimer, poly(ethyleneimine), and several cationic lipids (DOTAP, DC-Chol/DOPE, DOGS/DOPE, and DOTMA/DOPE). These agents activate the complement system to varying extents. Strong complement activation is seen with long-chain polylysines, the dendrimer, poly(ethyleneimine), and DOGS (half-maximal at about 3 microM amine content in the assay used). Compared to these compounds, the other cationic lipids (in **liposome** formulations) are weak activators of the complement system (half-maximal approximately 50-100 microM positive charge in assay). Complement activation by polylysine is strongly dependent on the chain length. Short-chain oligolysines are comparable to cationic lipids in their activation of complement. Incubation of these compounds with DNA to form complexes reduces complement activation in virtually all cases. The degree of complement activation by DNA complexes is strongly dependent on the ratio of polycation and DNA (expressed as the charge ratio) for polylysine, dendrimer, poly(ethyleneimine), and DOGS. To a lesser degree, charge ratio also influences complement activation by monovalent cationic lipid-DNA complexes. For polylysine-DNA complexes, complement activation can be considerably reduced by modifying the surface of preformed DNA complexes with polyethyleneglycol (half-maximal approximately 20 microM amine content). The data suggests that, by appropriate formulation of DNA complexes, complement activation can be minimized or even avoided. These findings should facilitate the search for DNA complex formulations appropriate for reproducible intravenous gene **delivery**.

18/3,AB/13 (Item 13 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08797781 96381784

Gene transfer in hepatocarcinoma cell lines: *in vitro* optimization of a virus-free system.

Ghoumari AM; Rixe O; Yarchoan SV; Zerrouqi A; Mouawad R; Poinard T; Opolon P; Khayat D; Soubrane C

Laboratoire du Service d'Oncologie Medicale, Hopital de la

Pitie-Salpetriere, Paris France.
Gene Ther (ENGLAND) Jun 1996, 3 (6) p483-90, ISSN 0969-7128
Journal Code: CCE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Many approaches exist for hepatic gene **delivery**, including viral vectors and non-viral vectors. In this study, we tested a panel of liposomes to transfer pAGO, a plasmid containing one copy of herpes simplex virus (HSVtk) gene, and pYED11, a plasmid containing two copies of the HSVtk gene, into a murine hepatocarcinoma cell line (Hepa 1-6) and a human hepatocarcinoma cell line (Hep-G2). The efficiency of gene **delivery** and expression was characterized by beta-galactosidase staining, flow cytometric analysis and quantitative lacZ activity. Different combinations of liposomes and DNA and the ratio of the concentration of **liposome** to DNA were tested. The efficient transfer was shown with **DOTAP** followed by transfectam and lipofectamine. Under these conditions, we tested the cytotoxicity of ganciclovir (GCV) exposure on Hepa 1-6 and Hep-G2 transfected separately with **liposome**-pAGO and **liposome**-pYED11 complexes. This study demonstrates the in vitro efficacy of each **liposome** tested to transduce the HSVtk gene into hepatocarcinoma cell lines. The transfer of two copies of the HSVtk gene rendered cells 1.5 times more sensitive to GCV than cells transduced by pAGO as compared to controls. This was achieved most efficiently by the **DOTAP**-pYED11 complex. Thus, pYED11 may be considered as an alternative to pAGO as a gene transfer vector.

18/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155: MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

08626905 96050905

Intratracheal gene **delivery** to the mouse airway: characterization of plasmid DNA expression and pharmacokinetics.

Meyer KB; Thompson MM; Levy MY; Barron LG; Szoka FC Jr
School of Pharmacy, University of California, San Francisco 94143-0446,
USA.

Gene Ther (ENGLAND) Sep 1995, 2 (7) p450-60, ISSN 0969-7128
Journal Code: CCE

Contract/Grant No.: DK 46052, DK, NIDDK; HL07192, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Intratracheal administration of plasmid DNA resulted in gene expression in mouse airways in the absence of any enhancing agent. Administration of plasmid DNA encoding the chloramphenicol acetyltransferase gene (CAT) in sterile water lead to CAT transgene expression that peaked between 1 and 3 days and was detected up to 28 days after DNA administration. Transgene expression was independent of mouse gender, age and strain. Levels of expression from DNA in various isotonic solutions did not differ from levels obtained with DNA administered in water, suggesting that transfection is not dependent on damage to airway cells caused by a hypo-osmotic **delivery** vehicle. Pharmacokinetic studies using radiolabeled plasmid DNA showed that DNA was rapidly degraded, while higher levels of radioactivity were retained for longer duration following administration of cationic **liposome**-DNA complexes in the airway. Southern blot and PCR analysis confirmed that DNA complexed with DOTMA-DOPE was retained in the airways for a longer period. However, cationic liposomes DOTMA-DOPE (1:1) or **DOTAP** complexed with DNA, did not enhance expression over DNA alone. These results suggest that 'naked' plasmid DNA should be included as a control in all studies on intratracheal gene **delivery** using nonviral systems.

18/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155: MEDLINE(R)

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08551207 96177661

Liposome-mediated gene transfer and expression via the skin.

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Department of Medical Genetics, Glasgow University, Yorkhill Hospital, UK.

Hum Mol Genet (ENGLAND) Dec 1995, 4 (12) p2279-85, ISSN 0964-6906
Journal Code: BRC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A beta-galactosidase gene expression construct was used to investigate the effectiveness of gene **delivery** and expression when DNA/**liposome** complexes were topically applied to mouse skin *in vivo*. DNA was complexed with commercial preparation of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl-ammonium-methyl-sulfate (**DOTAP**) in a ratio of 1:1.6 (w/w). The DNA rapidly penetrated the skin and was expressed in the epidermis, dermis and hair follicles. A DNA concentration of 267 microgram/ml DNA was found to be optimal for efficient transfection. Expression was seen as early as 6 h post-application, persisted at high levels 24 and 48 h post-treatment, but was markedly reduced by 7 days after application. In conclusion, utilising a commercially available **liposome** preparation, topically-applied DNA/**liposome** complexes can be efficiently **delivered** and expressed in several cell types within the skin. This simple, non-invasive technique may have implications for a number of gene therapy applications.

18/3,AB/16 (Item 16 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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08513018 96129240

Evaluation *in vitro* and *in vivo* of cationic **liposome**-expression construct complexes for cystic fibrosis gene therapy.

McLachlan G; Davidson DJ; Stevenson BJ; Dickinson P; Davidson-Smith H; Dorin JR; Porteous DJ

MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland.

Gene Ther (ENGLAND) Nov 1995, 2 (9) p614-22, ISSN 0969-7128

Journal Code: CCE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have tested the cationic **liposome** N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethyl-ammoniummethylsulfate, (**DOTAP**), for gene **delivery** *in vitro* and *in vivo* with a view to clinical use in gene therapy for cystic fibrosis. **Delivery** of lacZ cDNA-**DOTAP** complexes via aerosol showed promoter-dependent differences in the pattern and longevity of expression. Repeated administration was well tolerated. The potential for the transfer of foreign genes into reproductive tissue was investigated by intravenous injection of DNA-**DOTAP** into female mice. Foreign DNA was undetectable in the ovaries by Southern blot analysis at 1 and 7 days after injection. Our results suggest that **DOTAP** merits testing in cystic fibrosis patients for **delivery** of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to the respiratory tract and that substitution of the cytomegalovirus (CMV) promoter for the simian virus (SV) promoter may improve on the transitory response reported previously.

18/3,AB/17 (Item 17 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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08010858 95002064

Oligonucleotide-cationic **liposome** interactions. A physicochemical

study.

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Department of Pharmaceutical Technology, University of Kuopio, Finland.

Biochim Biophys Acta (NETHERLANDS) Oct 12 1994, 1195 (1) p115-23,

ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Cationic liposomes are effective in delivering antisense oligonucleotides into cells in culture, but their interactions with the oligonucleotides are poorly understood. We studied the aggregation and fusion reactions during the formation of cationic lipid/oligonucleotide complexes in solution and their interactions with lipid bilayers. Phosphorothioate oligonucleotides (15-mer) were complexed with cationic liposomes composed of dimethyldioctadecylammonium bromide (DDAB) and dioleoylphosphatidylethanolamine (DOPE) at 8:15 molar ratio or of a commercial formulation DOTAP (N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammoniummethylsulfate), at different ratios with apparent -/+ charge ratios of 0.03-5.6. Mean size of the complexes increased with -/+ ratio so that at charge ratios 0.4-2.0 the size increased by at least an order of magnitude due to the oligonucleotide induced aggregation. Resonance energy transfer experiments showed that in addition to aggregation oligonucleotides induced fusion of cationic liposomes, but the fusion was rate-controlled by the initial aggregation step. Rate constants for oligonucleotide induced aggregation were dependent on lipid concentration and were in the range of (0.2-1).10(7) M-1 s-1 and (1-10).10(7) M-1 s-1 for DDAB/DOPE and DOTAP, respectively. Increase in oligonucleotide concentration induced the aggregation and fusion until at high -/+ ratios electrostatic repulsion of negative surfaces inhibited further aggregation and fusion. DOTAP/oligonucleotide complexes did not induce leakage of calcein from neutral EPC liposomes, but did cause leakage at -/+ charge ratios of < 0.7 and > 2.0 from EPC/DOPE liposomes. Also at -/+ charge ratios below 0.8 DOTAP/oligonucleotide complexes induced leaking from negatively charged DPPC/DPPG liposomes. These results indicate that either phosphatidylethanolamine or negative charge are required in the cell membrane for fusion of cationic liposome-oligonucleotide complexes. The ratio of oligonucleotide to cationic lipid is critical in determining the physicochemical properties of the mixture.

18/3, AB/18 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11692228 BIOSIS NO.: 199800473959
Delivery of an anti-HIV-1 ribozyme into HIV-infected cells via cationic liposomes.

AUTHOR: Konopka Krystyna; Rossi John J; Swiderski Piotr; Slepushkin Vladimir A; Duzgunes Nejat(a)

AUTHOR ADDRESS: (a)Dep. Microbiol., Sch. Dent., Univ. Pacific, 2155 Webster St., San Francisco, CA 94115-2399, USA

JOURNAL: Biochimica et Biophysica Acta 1372 (1):p55-68 June 24, 1998
ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Cationic liposome-mediated intracellular delivery of a fluorescein-labeled chimeric DNA-RNA ribozyme targeted to the HIV-1 5' LTR was investigated, using THP-1, THP-1/HIV-1IIIB or HeLa/LAV cells. Different fluorescence patterns were observed when the cells were exposed to Lipofectamine, Lipofectin or DMRIE:DOPE (1:1) complexed to the ribozyme. With Lipofectamine intense cell-associated fluorescence was found. Incubation with Lipofectin resulted in less intense diffuse

fluorescence, while with DMRIE an intense but sporadic fluorescence was observed. Different lipid THP-1/HIV-1IIIB cells were more susceptible to killing by **liposome**-ribozyme complexes than TBP-1 cells. Under non-cytotoxic conditions (a 4-h treatment) complexes of 5, 10 or 15 μ M Lipofectin or **DOTAP**:DOPE (1:1) and ribozyme, at lipid:ribozyme ratios of 8:1 or 4:1, did not affect p24 production in THP-1/HIV-1IIIB cells in spite of the intracellular accumulation of the ribozyme. A 24-h exposure of THP-1/HIV-1IIIB cells to 5 μ M Lipofectin or **DOTAP**:DOPE (1:1) complexed with either the functional or a modified control ribozyme reduced virus production by approximately 30%. Thus, the antiviral effect of the **liposome**-complexed ribozyme was not sequence-specific. In contrast, the free ribozyme at a relatively high concentration inhibited virus production by 30%, while the control ribozyme was ineffective, indicating a sequence-specific effect. Both Lipofectin and **DOTAP** complexed with ribozyme were toxic at 10 and 15 μ M after a 24-h treatment. A 4-h treatment of HeLa/LAV cells with Lipofectin at 5, 10 or 15 μ M was not toxic to the cells, but also did not inhibit p24 production. In contrast, treatment of HeLa CD4+ cells immediately after infection with HIV-1IIIB at the same lipid concentrations and lipid:ribozyme ratios was cytotoxic. Our results indicate that the **delivery** of functional ribozyme into cells by cationic liposomes is an inefficient process and needs extensive improvement before it can be used in ex vivo and in vivo applications.

18/3, AB/19 (Item 2 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11446355 BIOSIS NO.: 199800227687
Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice.

AUTHOR: Thurston Gavin(a); McLean John W; Rizen Michael; Baluk Peter; Haskell Amy; Murphy Thomas J; Hanahan Douglas; McDonald Donald M
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JOURNAL: Journal of Clinical Investigation 101 (7):p1401-1413 April 1, 1998

ISSN: 0021-9738

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: This study sought to determine whether angiogenic blood vessels in disease models preferentially bind and internalize cationic liposomes injected intravenously. Angiogenesis was examined in pancreatic islet cell tumors of RIP-Tag2 transgenic mice and chronic airway inflammation in Mycoplasma pulmonis-infected C3H/HeNCr mice. For comparison, physiological angiogenesis was examined in normal mouse ovaries. We found that endothelial cells in all models avidly bound and internalized fluorescently labeled cationic liposomes (1,2-dioleoyl-3-trimethylammonium-propane (**DOTAP**)/cholesterol or dimethyldioctadecyl ammonium bromide (**DDAB**)/cholesterol) or **liposome**-DNA complexes. Confocal microscopic measurements showed that angiogenic endothelial cells averaged 15-33-fold more uptake than corresponding normal endothelial cells. Cationic **liposome**-DNA complexes were also avidly taken up, but anionic, neutral, or sterically stabilized neutral liposomes were not. Electron microscopic analysis showed that 32% of gold-labeled liposomes associated with tumor endothelial cells were adherent to the luminal surface, 53% were internalized into endosomes and multivesicular bodies, and 15% were extravascular 20 min after injection. Our findings indicate that angiogenic endothelial cells in these models avidly bind and internalize

cationic liposomes and **liposome**-DNA complexes but not other types of liposomes. This differential uptake raises the possibility of using cationic liposomes to target diagnostic or therapeutic agents selectively to angiogenic blood vessels in tumors and sites of chronic inflammation.

18/3,AB/20 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11425124 BIOSIS NO.: 199800206456
Major limitations in the use of cationic liposomes for DNA delivery.

AUTHOR: Filion Mario C; Phillips Nigel C(a)
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JOURNAL: International Journal of Pharmaceutics (Amsterdam) 162 (1-2):p
159-170 March 20, 1998

ISSN: 0378-5173

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Liposomal vectors formulated with cationic lipids and the fusogenic phospholipid dioleyolphosphatidylethanolamine (DOPE) are usually used to target DNA inside mammalian cells. Since macrophages constitute the major site of **liposome** localisation after parenteral administration we felt it prudent to examine the effect of cationic liposomes on the production of several important immunoinflammatory modulators secreted by activated macrophages. In addition, we have evaluated the toxicity of different cationic **liposome** formulations towards phagocytic macrophages and non-phagocytic T-lymphocytes. Our results indicate that cationic liposomes are able to down-regulate the synthesis of the protein kinase C (PKC)-dependent mediators nitric oxide (NO), tumour necrosis factor-alpha (TNF-alpha) and prostaglandin E2 (PGE2) by activated macrophages after in vitro incubation under non-toxic conditions or after in vivo treatment, while the production of PKC-independent IL-6 is not modified. We have shown that cationic lipids possess potent anti-inflammatory activity in vivo. Prolonged incubation (> 3 h) of macrophages with cationic liposomes induced a high level of toxicity (ED₅₀ < 50 nmol/ml) that was not seen with non-phagocytic T-cells (ED₅₀ > 1000 nmol/ml). The rank order of toxicity was DOPE/dimethyldioctylammonium bromide (DDAB) >
DOPE/dioleyoltrimethylammonium propane (**DOTAP**) =
DOPE/dimethylaminooethanecarbamoyl cholesterol (DC-Chol) >
DOPE/dimyristoyltrimethylammonium propane. The replacement of DOPE by dipalmitoylphosphatidylcholine (DPPC) or the incorporation of dipalmitoylphosphatidylethanolamine-PEG2000 (DPPE-PEG2000) in DOPE/cationic lipids reduced the toxicity toward macrophages and restored the synthesis of PKC-dependent modulators. The incorporation of DNA, either as an antisense oligonucleotide (15-mers) or as the plasmid vector pBR322 (4363 bp), in cationic liposomes did not reduce these adverse effects. These results, in addition to the observation that cationic liposomes are extremely toxic following oral administration, indicate that DOPE/catonic lipid liposomes are not appropriate for DNA (or drug) delivery.

18/3,AB/21 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11324447 BIOSIS NO.: 199800105779
A potential gene therapy pitfall: The effects of endotoxin on cationic

liposome mediated plasmid delivery.

AUTHOR: Poxon S; Hughes J

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JOURNAL: Pharmaceutical Research (New York) 14 (11 SUPPL.):pS642 Nov., 1997

CONFERENCE/MEETING: Annual Meeting of the American Association of Pharmaceutical Scientists Boston, Massachusetts, USA November 2-6, 1997
SPONSOR: American Association of Pharmaceutical Scientists

ISSN: 0724-8741

RECORD TYPE: Citation

LANGUAGE: English

18/3,AB/22 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11270978 BIOSIS NO.: 199800052310

A modular lymphographic magnetic resonance imaging contrast agent: Contrast enhancement with DNA transfection potential.

AUTHOR: Wisner Erik R; Aho-Sharon Kimberly L; Bennett Michael J; Penn Sharron G; Lebrilla Carlito B; Nantz Michael H(a)

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JOURNAL: Journal of Medicinal Chemistry 40 (25):p3992-3996 Dec. 2, 1997

ISSN: 0022-2623

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A gadolinium-chelated liposomal contrast agent has been prepared, and magnetic resonance imaging (MRI) efficacy has been examined by indirect magnetic resonance lymphography. A lipidic N,N'-dimethylethylenediamine derivative (4) containing a 10,12-diyne-diacyl domain was treated with DTPA anhydride followed by GdCl₃ complexation. The complex was confirmed using MALDI spectrometry. An equimolar mixture of the Gd-chelate lipid and a commercially available diyne-PE was formulated as a **liposome** suspension and irradiated with UV light prior to imaging experiments. Subcutaneous injection of the liposomal gadolinium agent and subsequent MRI of rabbit axillary and popliteal lymph nodes revealed significant contrast enhancement up to 4 h postinjection. To explore the possibility of imaging a DNA transfection event, the gadolinium contrast mixture was formulated with the cationic transfection lipid **DOTAP** and complexed with the reporter gene encoding luciferase. DNA transfection studies on the NIH3T3 cell line confirmed the transfection activity of the dual-purpose contrast agent and exemplified the potential toward development of an imaging and DNA **delivery** vehicle.

18/3,AB/23 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11224990 BIOSIS NO.: 199800006322

Toxicity and immunomodulatory activity of liposomal vectors formulated with cationic lipids toward immune effector cells.

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AUTHOR ADDRESS: (a)Faculte Pharmacie, C.P. 6128 Succ. Centre-Ville, Univ.

Montreal, Montreal, PQ, Canada

JOURNAL: Biochimica et Biophysica Acta 1329 (2):p345-356 Oct. 23, 1997

ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Liposomal vectors formulated with cationic lipids (cationic liposomes) and fusogenic dioleoylphosphatidylethanolamine (DOPE) have potential for modulating the immune system by **delivering** gene or antisense oligonucleotide inside immune cells. The toxicity and the immunoadjuvant activity of cationic liposomes containing nucleic acids toward immune effector cells has not been investigated in detail. In this report, we have evaluated the toxicity of liposomes formulated with various cationic lipids towards murine macrophages and T lymphocytes and the human monocyte-like U937 cell line. The effect of these cationic liposomes on the synthesis of two immunomodulators produced by activated macrophages, nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha), has also been determined. We have found that liposomes formulated from DOPE and cationic lipids based on diacyltrimethylammonium propane (dioleoyl-, dimyristoyl-, dipalmitoyl-, distearyl-: **DOTAP**, DMTAP, DPTAP, DSTAP) or dimethyldioctadecylammonium bromide (DDAB) are highly toxic in vitro toward phagocytic cells (macrophages and U937 cells), but not towards non-phagocytic T lymphocytes. The rank order of toxicity was DOPE/DDAB > DOPE/**DOTAP** > DOPE/DMTAP > DOPE/DPTAP > DOPE/DSTAP. The ED50's for macrophage toxicity were < 10 nmol/ml for DOPE/DDAB, 12 nmol/ml for DOPE/**DOTAP**, 50 nmol/ml for DOPE/DMTAP, 400 nmol/ml for DOPE/DPTAP and > 1000 nmol/ml for DOPE/DSTAP. The incorporation of DNA (antisense oligonucleotide or plasmid vector) into the cationic liposomes marginally reduced their toxicity towards macrophages. Although toxicity was observed with cationic lipids alone, it was clearly enhanced by the presence of DOPE. The replacement of DOPE by dipalmitoylphosphatidylcholine (DPPC) significantly reduced **liposome** toxicity towards macrophages, and the presence of dipalmitoylphosphatidylethanolamine-PEG2000 (DPPE-PEG2000: 10 mol%) in the liposomes completely abolished this toxicity. Cationic liposomes, irrespective of their DNA content, downregulated NO and TNF-alpha synthesis by lipopolysaccharide (LPS)/interferon-gamma (IFN-gamma)-activated macrophages. The replacement of DOPE by DPPC, or the addition of DPPE-PEG2000, restored NO and TNF-alpha synthesis by activated macrophages. Since macrophages constitute the major site of **liposome** localization after parenteral administration and play an important role in the control of the immune system, cationic liposomes should be used with caution to **deliver** gene or antisense oligonucleotide to mammalian cells. Cationic lipids show in vitro toxicity toward phagocytic cells and inhibit in vitro and in situ NO and TNF-alpha production by activated macrophages.

18/3, AB/24 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11183974 BIOSIS NO.: 199799805119
Anti-inflammatory activity of cationic lipids.

AUTHOR: Filion Mario C; Phillips Nigel C(a)

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JOURNAL: British Journal of Pharmacology 122 (3):p551-557 1997

ISSN: 0007-1188

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: 1. The effect of **liposome** phospholipid composition has been assumed to be relatively unimportant because of the presumed inert nature of phospholipids. 2. We have previously shown that cationic **liposome** formulations used for gene therapy inhibit, through their cationic component, the synthesis by activated macrophages of the pro-inflammatory mediators nitric oxide (NO) and tumour necrosis factor-alpha (TNF-alpha). 3. In this study, we have evaluated the ability of different cationic lipids to reduce footpad inflammation induced by carrageenan and by sheep red blood cell challenge. 4. Parenteral (i.p. or s.c) or local injection of the positively charged lipids dimethyldioctadecylammonium bromide (DDAB), dioleyoltrimethylammonium propane (**DOTAP**), dimyristoyltrimethylammonium propane (DMTAP) or dimethylaminoethanecarbamoyl cholesterol (DC-Chol) significantly reduced the inflammation observed in both models in a dose-dependent manner (maximum inhibition: 70-95%). 5. Cationic lipids associated with dioleyol- or dipalmitoyl-phosphatidylethanolamine retained their antiinflammatory activity while cationic lipids associated with dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylglycerol (DMPG) showed no anti-inflammatory activity, indicating that the release of cationic lipids into the macrophage cytoplasm is a necessary step for anti-inflammatory activity. The anti-inflammatory activity of cationic lipids was abrogated by the addition of dipalmitoylphosphatidylethanolamine-poly(ethylene)glycol-2000 (DPPE-PEG-2000) which blocks the interaction of cationic lipids with macrophages. 6. Because of the significant role of protein kinase C (PKC) in the inflammatory process we have determined whether the cationic lipids used in this study inhibit PKC activity. The cationic lipids significantly inhibited the activity of PKC but not the activity of a non-related protein kinase, PKA. The synthesis of interleukin-6 (IL-6), which is not dependent on PKC activity for its induction in macrophages, was not modified in vitro or in situ by cationic lipids. The synthesis of NO and TNF-alpha in macrophages, both of which are PKC-dependent, was downregulated by cationic lipids. 7. These results demonstrate that cationic lipids can be considered as novel anti-inflammatory agents. The downregulation of pro-inflammatory mediators through interaction of cationic lipids with the PKC pathway may explain this anti-inflammatory activity. Furthermore, since cationic lipids have intrinsic anti-inflammatory activity, cationic liposomes should be used with caution to **deliver** nucleic acids for gene therapy *in vivo*.

18/3, AB/25 (Item 8 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11131783 BIOSIS NO.: 199799752928
Overcoming the inhibitory effect of serum on lipofection by increasing the charge ratio of cationic **liposome** to DNA.

AUTHOR: Yang J-P; Huang L(a)
AUTHOR ADDRESS: (a)Lab. Drug Targeting, Dep. Pharmacol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261, USA

JOURNAL: Gene Therapy 4 (9):p950-960 1997
ISSN: 0969-7128
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Since cationic **liposome** was first developed as a lipofection reagent, a drawback has been noted in that the efficiency of lipofection decreases dramatically after addition of serum to the lipofection medium. This drawback hampers the application of cationic **liposome** for systematic **delivery** of genes. In the present studies, we found that the effect of serum on DC-chol **liposome**

-mediated lipofection is dependent on the charge ratio of liposome to DNA. Serum inhibited lipofection activity of the lipoplex at low charge ratios, whereas it enhanced, the lipofection activity at high charge ratios. This phenomenon was observed using DOTAP/DOPE but not lipofectamine. Measurement of cellular association of DNA showed that serum could reduce the binding of lipoplex to cells at all tested charge ratios, ie 0-10.6. Removal of negatively charged proteins from serum by DEAE-Sephadex column abolished the inhibitory effect of serum on lipofection. The fraction contained only negatively charged serum proteins which strongly inhibited lipofection at low charge ratios but not at higher charge ratios. Furthermore, preincubation of serum with positively charged polylysine, which neutralized negatively charged serum proteins, eliminated the inhibitory effect of serum on lipofection. In summary, inactivation of cationic liposome by serum is due to negatively charged serum proteins and it can be overcome by increasing charge ratio of cationic liposome-DNA lipoplexes or by neutralizing the serum with polylysine.

18/3,AB/26 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11069773 BIOSIS NO.: 199799690918
Cationic **liposome**-mediated gene **delivery** via systemic administration.

AUTHOR: Liu Dexi
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Pittsburgh, PA 15261, USA

JOURNAL: Journal of Liposome Research 7 (2-3):p187-205 1997
ISSN: 0898-2104
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cationic liposomes have been studied as a potential carrier for **delivering** genes to cells for the purpose of gene therapy. This report summarizes our efforts to characterize the *in vivo* expression of transgene **delivered** by cationic liposomes via intravenous administration. Using a CMV driven gene expression system containing cDNA of luciferase or green fluorescence protein gene as a reporter and two commonly used cationic lipids, 2, 3-dioleoyloxypropyl-1-trimethyl ammonium chloride (DOTMA) and 2, 3-dioleoyloxy-1-trimethylammonium propyl chloride (DOTAP), we demonstrate that a significant level of gene expression can be obtained in different organs including the lung, heart, spleen, liver and kidneys following intravenous administration in the mouse. Our finding show that the transfection efficiency of cationic liposomes is determined by the structure of the cationic lipids, the lipid composition of liposomes and cationic lipid to DNA ratio. Furthermore, gene expression was short in duration, peaked between 4-24 hours post injection, and dropped to less than 1% of the peak level within a 4 day period. Experiments with repeated injections revealed that cells initially transfected by the first transfection were not fully responsive to the subsequent second transfection for approximately 14 days.

18/3,AB/27 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11069426 BIOSIS NO.: 199799690571
Protamine sulfate provides enhanced and reproducible intravenous gene transfer by cationic **liposome**/DNA complex.

Department of Pharmacology and Therapeutics, University of British Columbia, Vancouver, Canada.

Biochemistry (UNITED STATES) Feb 27 1996, 35 (8) p2610-7, ISSN 0006-2960 Journal Code: A0G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The influence of poly(ethylene glycol)-lipid conjugates on phospholipid polymorphism has been examined using 31P-NMR and freeze--fracture electron microscopy. An equimolar mixture of dioleoylphosphatidylethanolamine (DOPE) and cholesterol adopts the hexagonal (HII) phase when hydrated under physiological conditions but can be stabilized in a bilayer conformation when a variety of PEG-lipid conjugates are included in the lipid mixture. These PEG conjugates produced an increase in the bilayer to hexagonal (HII) phase transition temperature and a broadening of the temperature range over which both phases coexisted. Further, the fraction of phospholipid adopting the bilayer phase increased with increasing mole fraction of PEG-lipid such that at 20 mole % DOPE--PEG2000 no HII phase phospholipid was observed up to a least 60 degrees C. Increasing the size of the PEG moiety from 2000 to 5000 Da (while maintaining the PEG-lipid molar ratio constant) increased the proportion of lipid in the bilayer phase. In contrast, varying the acyl chains of the PE anchor had no effect on polymorphic behavior. PEG-lipid conjugates in which ceramide provides the hydrophobic anchor also promoted bilayer formation in DOPE:cholesterol mixtures but at somewhat higher molar ratios compared to the corresponding PEG-PE species. The slightly greater effectiveness of the PE conjugates may result from the fact that these derivatives also possess a net negative charge. Phosphorus NMR spectroscopy indicated that a proportion of the phospholipid in DOPE:cholesterol:PEG --PE mixtures experienced isotropic motional averaging with this proportion being sensitive to both temperature and PEG molecular weight. Surprisingly, little if any isotropic signal was observed when PEG-ceramide was used in place of PEG-PE. Consistent with the 31P-NMR spectra, freeze-fracture electron microscopy showed the presence of small vesicles (diameter <200 nm) and lipidic particles in DOPE:cholesterol mixtures containing PEG-PE. We conclude that the effects of PEG-lipid conjugates on DOPE:cholesterol mixtures are 2-fold. First, the complementary "inverted cone" shape of the conjugate helps to accommodate the "cone-shaped" lipids, DOPE and cholesterol, in the bilayer phase. Second, the steric hindrance caused by the PEG group inhibits close apposition of bilayers, which is a prerequisite for the bilayer to HII phase transition.

20/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155: MEDLINE(R)
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04524977 85006684

Enzyme therapy: II. Effect of covalent attachment of polyethylene glycol on biochemical parameters and immunological determinants of beta-glucosidase and alpha-galactosidase.

Wieder KJ; Davis FF

J Appl Biochem (UNITED STATES) Aug-Oct 1983, 5 (4-5) p337-47, ISSN 0161-7354 Journal Code: HEA

Contract/Grant No.: GM-20946, GM, NIGMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The covalent attachment of polyethylene glycol (PEG) to beta-glucosidase from sweet almonds and alpha-galactosidase from green coffee beans results in alterations of their catalytic properties and masking of specific determinant sites on the enzymes. Both enzymes have increased Km and decreased Vmax values against their respective p-nitrophenyl substrate analogs after PEG attachment. When PEG is attached to 30% of alpha-galactosidase epsilon-amino groups, 12% activity remains against ceramide trihexoside, while its ability to

| Set | Items | Description |
|-----|-------|---|
| S1 | 501 | REVIEW AND LIPOSOME? |
| S2 | 0 | S1 AND PEG (2A) CERAMIDE |
| S3 | 0 | PEG (2A) CERAMIDE |
| S4 | 0 | S1 AND PEG AND CERAMIDE |
| S5 | 5 | S1 AND PEG |
| S6 | 4 | RD (unique items) |
| S7 | 0 | S1 AND CERAMIDE |
| S8 | 0 | S1 AND DODAC |
| S9 | 0 | S1 AND DOTAP |
| S10 | 0 | DODAC AND DOTAP AND LIPID AND GENE AND DELIVER? |
| S11 | 0 | DODAC AND DOTAP AND DELIVER? |
| S12 | 8 | DODAC AND DELIVER? |
| S13 | 5 | RD (unique items) |
| S14 | 93 | DOTAP AND DELIVER? |
| S15 | 91 | S14 NOT PY>1998 |
| S16 | 0 | L15 AND CHOLESTEROL |
| S17 | 0 | L15 AND PHOPHATIDYL CHOLINE |
| S18 | 40 | S15 AND LIPOSOME |

7/97 fol.

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18/3,AB/1 (Item 1 from file: 155)
 DIALOG(R) File 155: MEDLINE(R)
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09579424 98316152

Delivery of an anti-HIV-1 ribozyme into HIV-infected cells via cationic liposomes.

Konopka K; Rossi JJ; Swiderski P; Slepushkin VA; Duzgunes N
 Department of Microbiology, School of Dentistry, University of the Pacific, San Francisco, CA 94115, USA.

Biochim Biophys Acta (NETHERLANDS) Jun 24 1998, 1372 (1) p55-68,
 ISSN 0006-3002 Journal Code: AOW

Contract/Grant No.: AI-35231, AI, NIAID; AI-32399, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Cationic **liposome**-mediated intracellular **delivery** of a fluorescein-labeled chimeric DNA-RNA ribozyme targeted to the HIV-1 5' LTR was investigated, using THP-1, THP-1/HIV-1IIIB or HeLa/LAV cells. Different fluorescence patterns were observed when the cells were exposed to Lipofectamine, Lipofectin or DMRIE:DOPE (1:1) complexed to the ribozyme. With Lipofectamine intense cell-associated fluorescence was found. Incubation with Lipofectin resulted in less intense diffuse fluorescence, while with DMRIE an intense but sporadic fluorescence was observed. Differentiated THP-1/HIV-1IIIB cells were more susceptible to killing by **liposome**-ribozyme complexes than THP-1 cells. Under non-cytotoxic conditions (a 4-h treatment) complexes of 5, 10 or 15 microM Lipofectin or **DOTAP**:DOPE (1:1) and ribozyme, at lipid:ribozyme ratios of 8:1 or 4:1, did not affect p24 production in THP-1/HIV-1IIIB cells in spite of the intracellular accumulation of the ribozyme. A 24-h exposure of THP-1/HIV-1IIIB cells to 5 microM Lipofectin or **DOTAP**:DOPE (1:1) complexed with either the functional or a modified control ribozyme reduced virus production by approximately 30%. Thus, the antiviral effect of the **liposome**-complexed ribozyme was not sequence-specific. In contrast, the free ribozyme at a relatively high concentration inhibited virus production by 30%, while the control ribozyme was ineffective, indicating a

sequence-specific effect. Both Lipofectin and DOTAP complexed with ribozyme were toxic at 10 and 15 microM after a 4-h treatment. A 4-h treatment of HeLa/LAV cells with Lipofectin at 5, 10 or 15 microM was not toxic to the cells, but also did not inhibit p24 production. In contrast, treatment of HeLa CD4+ cells immediately after infection with HIV-1IIIB at the same lipid concentrations and lipid:ribozyme ratios was cytotoxic. Our results indicate that the delivery of functional ribozyme into cells by cationic liposomes is an inefficient process and needs extensive improvement before it can be used in ex vivo and in vivo applications. Copyright 1998 Elsevier Science B.V. All rights reserved.

18/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09557797 98267043
Nuclear localization signal peptides enhance cationic **liposome**-mediated gene therapy.
Aronsohn AI; Hughes JA
Department of Pharmaceutics, University of Florida, Gainesville 32610, USA.

J Drug Target (SWITZERLAND) 1998, 5 (3) p163-9, ISSN 1061-186X
Journal Code: B3S

Contract/Grant No.: R29 HL55770-02, HL, NHLBI; P01 AG10485-06, AG, NIA
Languages: ENGLISH

Document type: JOURNAL ARTICLE

The use of genes as therapeutic drugs will likely involve non-viral **delivery** systems. While traditionally less effective for gene expression, the advantages of a non-viral **delivery** system include ease of production, lower toxicity, and no risk of infection. However, most non-viral systems do not incorporate a mechanism for gene transport into the nucleus. Nuclear localization signal peptides can combine the increased expression of viral **delivery** systems with the safety and ease of preparation of non-viral **delivery** systems. A novel non-viral **delivery** vehicle consisting of a conglomerate of a synthetic nuclear localization signal peptide derived from the SV40 virus, a luciferase encoding PGL3 plasmid, and a cationic lipid DOTAP:DOPE (1:1 w/w) **liposome** was transfected into SKnSH mammalian neuroblastoma cells. A three-fold increase in luciferase expression was seen with the **delivery** system containing a NLS peptide over cationic **liposome** controls. Examination of the factors that limit the rate of transgene expression can potentially lead to the discovery of new ways to improve the efficiency and efficacy of nonviral methods of gene therapy.

18/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09551643 98288300
Cationic liposomes coated with polyethylene glycol as carriers for oligonucleotides.

Meyer O; Kirpotin D; Hong K; Sternberg B; Park JW; Woodle MC; Papahadjopoulos D

Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, California 94143, USA.

J Biol Chem (UNITED STATES) Jun 19 1998, 273 (25) p15621-7, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: P50CA58207, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Modification of **liposome** surface with polyethylene glycol was used to improve oligodeoxyribonucleotide (ODN) loading, stability of the resulting complexes, and specificity of cellular **delivery** of ODN by

cationic liposomes. Liposomes composed of a cationic lipid (**DOTAP**, DOGS, DDAB), a neutral lipid (DOPE), and a phospholipid derivative of polyethylene glycol (PEG-PE) formed a complex with 18-mer phosphorothioate up to ODN/lipid molar ratio of 0.25. The complexes showed intact vesicular structures similar to original liposomes and their size (100–130 nm) was unchanged after several weeks of storage, whereas complexes lacking PEG-PE showed progressive aggregation and/or precipitation. After exposure to human plasma, PEG-modified cationic liposomes retained over 60% of the originally bound ODN. PEG-coated complexes resulted in 4–13-fold enhancement of the ODN uptake by human breast cancer cells in serum-supplemented growth medium, relative to free ODN. Complexes containing conjugated anti-HER2 F(ab') fragments at the distal termini of PEG chains efficiently **delivered** ODN primarily into the cytoplasm and nuclei of HER2 overexpressing cancer cells and greatly enhanced the biological activity of antisense ODN. The development of PEG-modified cationic liposomes may lead to improved ODN potency *in vivo*.

18/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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09348818 98069687
A modular lymphographic magnetic resonance imaging contrast agent:
contrast enhancement with DNA transfection potential.
Wisner ER; Aho-Sharon KL; Bennett MJ; Penn SG; Lebrilla CB; Nantz MH
Department of Chemistry, University of California, Davis 95616, USA.
J Med Chem (UNITED STATES) Dec 5 1997, 40 (25) p3992-6, ISSN
0022-2623 Journal Code: J0F
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A gadolinium-chelated liposomal contrast agent has been prepared, and magnetic resonance imaging (MRI) efficacy has been examined by indirect magnetic resonance lymphography. A lipidic N,N'-dimethylethylenediamine derivative (4) containing a 10,12-diyne-diacyl domain was treated with DTPA anhydride followed by GdCl₃ complexation. The complex was confirmed using MALDI spectrometry. An equimolar mixture of the Gd-chelate lipid and a commercially available diyne-PE was formulated as a **liposome** suspension and irradiated with UV light prior to imaging experiments. Subcutaneous injection of the liposomal gadolinium agent and subsequent MRI of rabbit axillary and popliteal lymph nodes revealed significant contrast enhancement up to 4 h postinjection. To explore the possibility of imaging a DNA transfection event, the gadolinium contrast mixture was formulated with the cationic transfection lipid **DOTAP** and complexed with the reporter gene encoding luciferase. DNA transfection studies on the NIH3T3 cell line confirmed the transfection activity of the dual-purpose contrast agent and exemplified the potential toward development of an imaging and DNA **delivery** vehicle.

18/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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09316152 98011879
Anti-inflammatory activity of cationic lipids.
Filion MC; Phillips NC
Faculte de Pharmacie, Universite de Montreal, Quebec, Canada.
Br J Pharmacol (ENGLAND) Oct 1997, 122 (3) p551-7, ISSN 0007-1188
Journal Code: B00
Languages: ENGLISH
Document type: JOURNAL ARTICLE
1. The effect of **liposome** phospholipid composition has been assumed to be relatively unimportant because of the presumed inert nature of phospholipids. 2. We have previously shown that cationic **liposome**

formulations used for gene therapy inhibit, through their cationic component, the synthesis by activated macrophages of the pro-inflammatory mediators nitric oxide (NO) and tumour necrosis factor-alpha (TNF-alpha). 3. In this study, we have evaluated the ability of different cationic lipids to reduce footpad inflammation induced by carrageenan and by sheep red blood cell challenge. 4. Parenteral (i.p. or s.c) or local injection of the positively charged lipids dimethyldioctadecylammonium bromide (DDAB), dioleyoltrimethylammonium propane (DOTAP), dimyristoyltrimethylammonium propane (DMTAP) or dimethylaminoethanecarbamoyl cholesterol (DC-Chol) significantly reduced the inflammation observed in both models in a dose-dependent manner (maximum inhibition: 70-95%). 5. Cationic lipids associated with dioleyol- or dipalmitoyl-phosphatidylethanolamine retained their anti-inflammatory activity while cationic lipids associated with dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylglycerol (DMPG) showed no anti-inflammatory activity, indicating that the release of cationic lipids into the macrophage cytoplasm is a necessary step for anti-inflammatory activity. The anti-inflammatory activity of cationic lipids was abrogated by the addition of dipalmitoylphosphatidylethanolamine-poly(ethylene)glycol-2000 (DPPE-PEG2000) which blocks the interaction of cationic lipids with macrophages. 6. Because of the significant role of protein kinase C (PKC) in the inflammatory process we have determined whether the cationic lipids used in this study inhibit PKC activity. The cationic lipids significantly inhibited the activity of PKC but not the activity of a non-related protein kinase, PKA. The synthesis of interleukin-6 (IL-6), which is not dependent on PKC activity for its induction in macrophages, was not modified in vitro or in situ by cationic lipids. The synthesis of NO and TNF-alpha in macrophages, both of which are PKC-dependent, was downregulated by cationic lipids. 7. These results demonstrate that cationic lipids can be considered as novel anti-inflammatory agents. The downregulation of pro-inflammatory mediators through interaction of cationic lipids with the PKC pathway may explain this anti-inflammatory activity. Furthermore, since cationic lipids have intrinsic anti-inflammatory activity, cationic liposomes should be used with caution to deliver nucleic acids for gene therapy in vivo.

18/3, AB/6 (Item 6 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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09312851 98037477

Toxicity and immunomodulatory activity of liposomal vectors formulated with cationic lipids toward immune effector cells.

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Biochim Biophys Acta (NETHERLANDS) Oct 23 1997, 1329 (2) p345-56,
ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Liposomal vectors formulated with cationic lipids (cationic liposomes) and fusogenic dioleyolphosphatidylethanolamine (DOPE) have potential for modulating the immune system by delivering gene or antisense oligonucleotide inside immune cells. The toxicity and the immunoadjuvant activity of cationic liposomes containing nucleic acids toward immune effector cells has not been investigated in detail. In this report, we have evaluated the toxicity of liposomes formulated with various cationic lipids towards murine macrophages and T lymphocytes and the human monocyte-like U937 cell line. The effect of these cationic liposomes on the synthesis of two immunomodulators produced by activated macrophages, nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha), has also been determined. We have found that liposomes formulated from DOPE and cationic lipids based on diacyltrimethylammonium propane (dioleyoyl-, dimyristoyl-, dipalmitoyl-, disteroyl-: DOTAP, DMTAP, DPTAP, DSTAP) or dimethyldioctadecylammonium bromide (DDAB) are highly toxic in vitro toward phagocytic cells (macrophages and U937 cells), but not towards

non-phagocytic T lymphocytes. The rank order of toxicity was DOPE/DDAB > DOPE/**DOTAP** > DOPE/DOPIP > DOPE/DPTAP > DOPE/DSTAP. The ED50's for macrophage toxicity were < 10 nmol/ml for DOPE/DDAB, 12 nmol/ml for DOPE/**DOTAP**, 50 nmol/ml for DOPE/DMTAP, 400 nmol/ml for DOPE/DPTAP and > 1000 nmol/ml for DOPE/DSTAP. The incorporation of DNA (antisense oligonucleotide or plasmid vector) into the cationic liposomes marginally reduced their toxicity towards macrophages. Although toxicity was observed with cationic lipids alone, it was clearly enhanced by the presence of DOPE. The replacement of DOPE by dipalmitoylphosphatidylcholine (DPPC) significantly reduced **liposome** toxicity towards macrophages, and the presence of dipalmitoylphosphatidylethanolamine-PEG2000 (DPPE-PEG2000: 10 mol%) in the liposomes completely abolished this toxicity. Cationic liposomes, irrespective of their DNA content, downregulated NO and TNF-alpha synthesis by lipopolysaccharide (LPS)/interferon-gamma (IFN-gamma)-activated macrophages. The replacement of DOPE by DPPC, or the addition of DPPE-PEG2000, restored NO and TNF-alpha synthesis by activated macrophages. Since macrophages constitute the major site of **liposome** localization after parenteral administration and play an important role in the control of the immune system, cationic liposomes should be used with caution to **deliver** gene or antisense oligonucleotide to mammalian cells. Cationic lipids show in vitro toxicity toward phagocytic cells and inhibit in vitro and in situ NO and TNF-alpha production by activated macrophages.

18/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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09286845 98010145

Overcoming the inhibitory effect of serum on lipofection by increasing the charge ratio of cationic **liposome** to DNA.

Yang JP; Huang L

Department of Pharmacology, University of Pittsburgh School of Medicine, PA 15261, USA.

Gene Ther (ENGLAND) Sep 1997, 4 (9) p950-60, ISSN 0969-7128

Journal Code: CCE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Since cationic **liposome** was first developed as a lipofection reagent, a drawback has been noted in that the efficiency of lipofection decreases dramatically after addition of serum to the lipofection medium. This drawback hampers the application of cationic **liposome** for systematic **delivery** of genes. In the present studies, we found that the effect of serum on DC-chol **liposome**-mediated lipofection is dependent on the charge ratio of **liposome** to DNA. Serum inhibited lipofection activity of the lipoplex at low charge ratios, whereas it enhanced the lipofection activity at high charge ratios. This phenomenon was observed using **DOTAP**/DOPE but not lipofectamine. Measurement of cellular association of DNA showed that serum could reduce the binding of lipoplex to cells at all tested charge ratios, i.e. 0-10.6. Removal of negatively charged proteins from serum by DEAE Sephadex column abolished the inhibitory effect of serum on lipofection. The fraction contained only negatively charged serum proteins which strongly inhibited lipofection at low charge ratios but not at higher charge ratios. Furthermore, preincubation of serum with positively charged polylysine, which neutralized negatively charged serum proteins, eliminated the inhibitory effect of serum on lipofection. In summary, inactivation of cationic **liposome** by serum is due to negatively charged serum proteins and it can be overcome by increasing charge ratio of cationic **liposome**-DNA lipoplexes or by neutralizing the serum with polylysine.

18/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155: MEDLINE(R)

formation was contingent on the incubation period after partitioning the DNA/lipid mixture into organic and aqueous phases. These results suggest that the cationic lipid/DNA complex forms at the aqueous/organic interface and that DNA/lipid binding is dependent on multivalent interactions at this interface. A Scatchard analysis of DNA/**DODAC** binding demonstrated that the binding reaction exhibits a high degree of positive cooperativity. The apparent dissociation constant (K_n), using data obtained under conditions where **DODAC** binding to DNA approached saturation, indicated a high-affinity reaction ($K_n > 10(-11)$ mol L $^{-1}$). At this point, approximately 8400 mol of **DODAC** was bound per mole of DNA, which is equivalent to a charge ratio (+/-) of 0.585 for the 7.2 kb plasmid used and suggests that formation of the hydrophobic complex occurs at a stage prior to charge neutralization. The influence of other lipids on DNA/cationic lipid binding at the aqueous/organic interface was also studied. Cholesterol and DOPC had little effect on DNA/**DODAC** binding while the anionic lipids LPI, DOPS, and DMPG inhibited complex formation. The zwitterionic lipid DOPE, however, had a concentration-dependent effect on cationic lipid binding that was also dependent on the mixing order. We believe that this approach for evaluating lipid/DNA binding provides an effective procedure for assessing factors which control the dissociation of lipids from DNA and may be beneficial in the selection of lipids for effective use in gene transfection studies.

13/3,AB/5 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11533060 BIOSIS NO.: 199800314392
Stabilization and regulated fusion of liposomes containing a cationic lipid using amphipathic polyethyleneglycol derivatives.

AUTHOR: Mori Atsuhide; Chonn Arcadio; Choi Lewis S; Israels Alix; Monck Myrna A(a); Cullis Pieter R
AUTHOR ADDRESS: (a)Inex Pharmaceuticals Corp., 100-8900 Glenlyon Pkwy.,
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JOURNAL: Journal of Liposome Research 8 (2):p195-211 May, 1998

ISSN: 0898-2104

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Current fusogenic liposomal **delivery** systems have limited applicability *in vivo* due to poor stability in the blood and rapid clearance from the circulation. This is particularly true for liposomes composed of dioleoylphosphatidylethanolamine (DOPE) and cationic lipids, currently being developed for systemic **delivery** of gene-based drugs. This paper describes a potential strategy to overcome these problems, involving the incorporation of exchangeable amphipathic polyethyleneglycol (PEG) derivatives to transiently stabilize fusogenic liposomes while in the circulation, but where the PEG coating dissipates to reveal fusogenic character at later times after arrival at target sites. It is shown here that large unilamellar vesicles (LUVs) containing DOPE and the cationic lipid, N,N-dioleoyl-N,N-dimethylammonium chloride (**DODAC**) can be stabilized against serum-induced aggregation and fusion by inclusion of at least 2 mol% of PEG coupled to phosphatidylethanolamine (PEG-PE) or ceramide (PEG-Cer). However, low *in vitro* recovery of fusogenic activity was obtained for the PEG-PE-containing system, presumably due to electrostatic interactions between the negatively charged PEG-PE and the cationic lipid which prevent PEG-PE dissociation from the LUV. Improved recovery of fusogenic activity was achieved for LUVs stabilized by the neutral PEG-Cer derivatives, with shorter chain ceramides exhibiting more rapid recovery rates. Biodistribution studies showed that DOPE/**DODAC** (85:15,

mol/mol) LUVs were rapidly cleared from the circulation, whereas inclusion of 10 mol% EG-Cer(C20) resulted in significantly prolonged circulation time. Inclusion of shorter ceramide chain lengths resulted in decreased circulation times, consistent with increased exchangeability. These findings demonstrate the feasibility of developing a cationic liposome that is stable in the circulation, but retains its ability to fuse with membranes. This work represents the first step toward the rationale design of fusogenic cationic liposomes for the systemic delivery of gene-based drugs to target tissues, such as tumors.

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S20 9 RD ique items)
? t s20/3,ab/all

20/3,AB/1 (Item 1 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09616312 98342124

Comparison of different hydrophobic anchors conjugated to poly(ethylene glycol): effects on the pharmacokinetics of liposomal vincristine.

Webb MS; Saxon D; Wong FM; Lim HJ; Wang Z; Bally MB; Choi LS; Cullis PR; Mayer LD

British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, B.C. V5Z 4E6, Canada.

Biochim Biophys Acta (NETHERLANDS) Jul 17 1998, 1372 (2) p272-82,
ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Poly(ethylene glycol) (**PEG**) conjugated lipids have been used to increase the circulation longevity of liposomal carriers encapsulating therapeutic compounds. **PEG** is typically conjugated to distearoylphosphatidylethanolamine (DSPE) via a carbamate linkage that results in a net negative charge on the phosphate moiety at physiological pH. It was anticipated that the presence of this negative charge could have deleterious effects on liposome pharmacokinetic characteristics. We describe here the synthesis of a new class of neutrally charged **PEG**-lipid conjugates in which the **PEG** moiety was linked to **ceramide** (CER). These **PEG**-CER conjugates were compared with **PEG**-DSPE conjugates for their effects on the pharmacokinetics of liposomal vincristine. **PEG**-CER (78% palmitic acid, C16) and **PEG**-DSPE achieved comparable increases in the circulation lifetimes of sphingomyelin/cholesterol (SM/chol) liposomes. However, **PEG**-DSPE significantly increased the *in vitro* and *in vivo* leakage rates of vincristine from SM/chol-based liposomes compared to vincristine leakage observed when **PEG**-CER was used. The increase in drug leakage observed *in vitro* that was due to the presence of **PEG**-DSPE was likely due to the presence of a negative surface charge. Analysis of the electrophoretic mobilities of these formulations suggested that the negative surface charges were shielded by approx. 80% by the **PEG** layer extending from the membrane surface. In contrast, formulations containing **PEG**-CER had no surface charge and no electrophoretic mobility. A comparison of the effects of the **ceramide** acyl chain length (C8 through C24) on the pharmacokinetics of SM/chol/**PEG**-CER formulations of vincristine demonstrated that longer acyl chains on the **PEG**-CER were associated with longer circulation lifetimes of the liposomal carriers and, consequently, higher plasma vincristine concentrations. These data suggest that the short chain **PEG**-ceramides underwent rapid partitioning from the vesicles after *i.v.* administration, whereas the longer chain **PEG**-ceramides had stronger anchoring properties in the liposome bilayers and partitioned slowly from the administered vesicles. These data demonstrate the utility of **ceramide**-based steric stabilizing lipids as well as the potential for developing controlled release formulations by manipulating the retention of the **PEG**-**ceramide** conjugate in liposome bilayers.

20/3,AB/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08674789 96180685

Poly(ethylene glycol)-lipid conjugates promote bilayer formation in mixtures of non-bilayer-forming lipids.

Holland JW; Cullis PR; Madden TD

convert type B erythrocytes to type H specificity is lost. However, it still is able to clear terminal galactose residues from human saliva blood group substance B. **PEG**-beta-glucosidase (38%) did not elicit the production of complement-fixing antibodies, nor did it react with antibodies produced against the native enzyme. Antibody and lectin-specific binding were lost from both modified enzymes (**PEG**-beta-glucosidase and **PEG**-alpha-galactosidase). After conjugation with **PEG**, beta-glucosidase lost its ability to bind to concanavalin A-Sepharose. Antibodies directed against native alpha-galactosidase blocked its enzyme activity, but lost their ability to inhibit activity in progressively higher modified preparations of the enzyme. Antisera against **PEG**-alpha-galactosidase (53%) did not inhibit enzyme activity in any alpha-galactosidase or **PEG**-alpha-galactosidase preparation. These results indicate that **PEG** tends to cover lectin-specific carbohydrate moieties and antigenic determinants and that these sites probably remain cryptic during in vivo processing of **PEG**-enzymes.

20/3,AB/4 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11533060 BIOSIS NO.: 199800314392
Stabilization and regulated fusion of liposomes containing a cationic lipid using amphipathic polyethyleneglycol derivatives.

AUTHOR: Mori Atsuhide; Chonn Arcadio; Choi Lewis S; Israels Alix; Monck Myrna A(a); Cullis Pieter R

AUTHOR ADDRESS: (a)Inex Pharmaceuticals Corp., 100-8900 Glenlyon Pkwy.,
Glenlyon Business Park, Burnaby, BC V5J 5J8, Canada

JOURNAL: Journal of Liposome Research 8 (2):p195-211 May, 1998

ISSN: 0898-2104

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Current fusogenic liposomal delivery systems have limited applicability in vivo due to poor stability in the blood and rapid clearance from the circulation. This is particularly true for liposomes composed of dioleoylphosphatidylethanolamine (DOPE) and cationic lipids, currently being developed for systemic delivery of gene-based drugs. This paper describes a potential strategy to overcome these problems, involving the incorporation of exchangeable amphipathic polyethyleneglycol (**PEG**) derivatives to transiently stabilize fusogenic liposomes while in the circulation, but where the **PEG** coating dissipates to reveal fusogenic character at later times after arrival at target sites. It is shown here that large unilamellar vesicles (LUVs) containing DOPE and the cationic lipid, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC) can be stabilized against serum-induced aggregation and fusion by inclusion of at least 2 mol% of **PEG** coupled to phosphatidylethanolamine (**PEG**-PE) or **ceramide** (**PEG**-Cer). However, low in vitro recovery of fusogenic activity was obtained for the **PEG**-PE-containing system, presumably due to electrostatic interactions between the negatively charged **PEG**-PE and the cationic lipid which prevent **PEG**-PE dissociation from the LUV. Improved recovery of fusogenic activity was achieved for LUVs stabilized by the neutral **PEG**-Cer derivatives, with shorter chain ceramides exhibiting more rapid recovery rates. Biodistribution studies showed that DOPE/DODAC (85:15, mol/mol) LUVs were rapidly cleared from the circulation, whereas inclusion of 10 mol% **PEG**-Cer(C20) resulted in significantly prolonged circulation time. Inclusion of shorter **ceramide** chain lengths resulted in decreased circulation times, consistent with increased exchangeability. These findings demonstrate the feasibility of developing a cationic liposome

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AUTHOR ADDRESS: Lab. Drug Targeting, Dep. Pharmacol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261, USA

JOURNAL: Journal of Liposome Research 7 (2-3):p207-219 1997
ISSN: 0898-2104
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A novel lipid/polycation/DNA (LPD) formulation has been developed for in gene transfer. It involves the condensation of plasmid DNA with protamine sulfate, a cationic polypeptide, followed by the addition of **DOTAP** cationic liposomes. Compared with **DOTAP**/DNA complex, LPD offers greater protection of plasmid DNA against enzymatic digestion and gives consistently higher gene expression in mice via tail vein injection. The in vivo efficiency of LPD was dependent upon charge ratio and was also affected by the lipid used. Increasing the amount of DNA **delivered** induced an increase in gene expression. The optimal dose was approximately 50 mu-g per mouse, at which concentration approximately 10 ng luciferase protein per mg extracted tissue protein could be detected in the lung. Gene expression in the lung was detected as early as 1 h after injection, peaked at 6 h, and declined thereafter. Using LacZ as a reporter gene, it was shown that endothelial cells were the primary locus of transgene expression in both lung and spleen. No sign of inflammation in these organs was noticed. Since protamine sulfate has been proven to be non-toxic and only weakly immunogenic in humans, this novel vector may be useful for the clinical use of gene therapy.

18/3, AB/28 (Item 11 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11022773 BIOSIS NO.: 199799643918
Improved DNA: **Liposome** complexes for increased systemic **delivery** and gene expression.

AUTHOR: Templeton Nancy Smyth(a); Lasic Danilo D; Frederik Peter M; Strey Helmut H; Roberts David D; Pavlakis George N
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JOURNAL: Nature Biotechnology 15 (7):p647-652 1997
ISSN: 1087-0156
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To increase cationic **liposome**-mediated intravenous DNA **delivery** extruded **DOTAP**:cholesterol liposomes were used to form complexes with DNA, resulting in enhanced expression of the chloramphenicol acetyltransferase gene in most tissues examined. The DNA:**liposome** ratio, and mild sonication, heating, and extrusion steps used for **liposome** preparation were crucial for improved systemic **delivery**. Size fractionation studies showed that maximal gene expression was produced by a homogeneous population of DNA:**liposome** complexes between 200 to 450 nm in size. Cryo-electron microscopy examination demonstrates that the DNA:**liposome** complexes have a novel morphology, and that the DNA is condensed on the interior of invaginated liposomes between two lipid bilayers. This structure could account for the high efficiency of gene **delivery** in vivo and for the broad tissue distribution of the DNA:**liposome** complexes. Ligands can be placed on the outside of this structure to provide for targeted gene **delivery**.

18/3,AB/29 (Item from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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10882041 BIOSIS NO.: 199799503186
Evidence for safety and efficacy of **DOTAP** cationic **liposome**
mediated CFTR gene transfer to the nasal epithelium of patients with
cystic fibrosis.

AUTHOR: Porteous D J(a); Dorin J R; McLachlan G; Davidson-Smith H; Davidson H; Stevenson B J; Carothers A D; Wallace W A H; Moralee S; Hoenes C; Kallmeyer G; Michaelis U; Naujoks K; Ho L-P; Samways J M; Imrie M; Greening A P; Innes J A

AUTHOR ADDRESS: (a)MRC Human Genetics Unit, Western General Hosp., Crewe Road, Edinburgh, EH4 2XU, UK

JOURNAL: Gene Therapy 4 (3):p210-218 1997

ISSN: 0969-7128

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: In cystic fibrosis (CF), mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene results in defective transepithelial ion transport, leading to life shortening inflammatory lung disease. Before lung studies, we tested the safety and efficacy of gene **delivery** to the nasal epithelium of CF patients using pCMV-CFTR-**DOTAP** cationic **liposome** complex. A single dose of 400 mu-g pCMV-CFTR:2.4 mg **DOTAP** was administered in a randomised, double-blinded fashion to the nasal epithelium of eight CF patients, with a further eight receiving buffer only. Patients were monitored for signs and symptoms for 2 weeks before treatment and 4 weeks after treatment. Inflammatory cells were quantified in a nasal biopsy taken 3 days after treatment. There was no evidence for excess nasal inflammation, circulating inflammatory markers or other adverse events ascribable to active treatment. Gene transfer and expression were assayed by the polymerase chain reaction. Transgene DNA was detected in seven of the eight treated patients up to 28 days after treatment and vector derived CFTR mRNA in two of the seven patients at +3 and +7 days. Transepithelial ion transport was assayed before and after treatment by nasal potential difference during drug perfusion and by SPQ fluorescence halide ion conductance. Partial, sustained correction of CFTR-related functional changes toward normal values were detected in two treated patients. The level of gene transfer and functional correction were comparable to those reported previously using adenoviral vectors or another DNA-**liposome** complex, but here were sustained and uncompromised by false positives. These results justify further studies with pCMV-CFTR-**DOTAP** aimed at treating CF lung disease.

18/3,AB/30 (Item 13 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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10834779 BIOSIS NO.: 199799455924
Enhanced gene **delivery** and expression in human hepatocellular carcinoma cells by cationic immunoliposomes.

AUTHOR: Compagnon Beatrice; Moradpour Darius; Alford Dennis R; Larsen Charles E; Stevenson Michael J; Mohr Leonhard; Wands Jack R; Nicolau Claude(a)

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JOURNAL: Journal of Liposome Research 7 (1):p127-141 1997

ISSN: 0898-2104
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A targeted vector allowing enhanced gene transfer to human hepatocellular carcinoma (HCC-1) cells in vitro was developed using cationic liposomes covalently conjugated with the mAb AF-20. This high affinity antibody recognizes a rapidly internalized 180 kDa cell surface glycoprotein which is abundantly expressed on the surface of human HCC and other cancer cells. Quantitative binding analysis of liposomes with target cells by flow cytometry showed specific association of mAb-targeted liposomes with human HCC cells. Using mAb-targeted cationic liposomes containing 20% DOTAP, in the presence or absence of serum, gene expression in HuH-7 cells was enhanced up to 40-fold as compared to liposomes conjugated with an isotype-matched nonrelevant control antibody. Transfection specificity was not observed in AF-20. This study demonstrates that cationic **liposome** formulations a control cell line that does not express the antigen recognized by mAb in vitro gene **delivery** and expression in the presence or absence of serum.

18/3,AB/31 (Item 14 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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10820858 BIOSIS NO.: 199799442003
Cationic **liposome** mediated drug **delivery**.

AUTHOR: Campbell Robert B; Straubinger Robert M
AUTHOR ADDRESS: Dep. Biophysics, Roswell Park Cancer Inst., Buffalo, NY,
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JOURNAL: Biophysical Journal 72 (2 PART 2):pA303 1997

CONFERENCE/MEETING: 41st Annual Meeting of the Biophysical Society New Orleans, Louisiana, USA March 2-6, 1997
ISSN: 0006-3495
RECORD TYPE: Citation
LANGUAGE: English

18/3,AB/32 (Item 15 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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10685961 BIOSIS NO.: 199799307106
Enhanced non-viral gene expression with the use of nuclear localization signal peptides.

AUTHOR: Aronsohn A I; Hughes J A
AUTHOR ADDRESS: Dep. Pharm., University Florida, Gainesville, FL 32610,
USA

JOURNAL: Pharmaceutical Research (New York) 13 (9 SUPPL.):pS384 1996

CONFERENCE/MEETING: Annual Meeting of the American Association of Pharmaceutical Scientists Seattle, Washington, USA October 27-31, 1996
ISSN: 0724-8741
RECORD TYPE: Citation
LANGUAGE: English

18/3,AB/33 (Item 16 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1

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10469453 BIOSIS NO.: 199699090598
Gene transfer in hepatocarcinoma cell lines: In vitro optimization of a virus-free system.

AUTHOR: Ghoumari A M(a); Rixe O; Yarovo S V; Zerrouqi A; Mouawad R;
Poinnard T; Opolon P; Khayat D; Soubrane C
AUTHOR ADDRESS: (a)Laboratoire du Service d'Oncologie Medicale, 47
Boulevard de l'Hopital, 75013 Paris, France

JOURNAL: Gene Therapy 3 (6):p483-490 1996

ISSN: 0969-7128

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Many approaches exist for hepatic gene **delivery**, including viral vectors and non-viral vectors. In this study, we tested a panel of liposomes to transfer pAG0, a plasmid containing one copy of herpes simplex virus (HSVtk) gene, and pYED11, a plasmid containing two copies of the HSVtk gene, into a murine hepatocarcinoma cell line (Hepa 1-6) and a human hepatocarcinoma cell line (Hep-G2). The efficiency of gene **delivery** and expression was characterized by beta-galactosidase staining, flow cytometric analysis and quantitative lacZ activity. Different combinations of liposomes and DNA and the ratio of the concentration of **liposome** to DNA were tested. The efficient transfer was shown with **DOTAP** followed by transfectam and lipofectamine. Under these conditions, we tested the cytotoxicity of ganciclovir (GCV) exposure on Hepa 1-6 and Hep-G2 transfected separately with **liposome**-pAG0 and **liposome**-pYED11 complexes. This study demonstrates the in vitro efficacy of each **liposome** tested to transduce the HSVtk gene into hepatocarcinoma cell lines. The transfer of two copies of the HSVtk gene rendered cells 1.5 times more sensitive to GCV than cells transduced by pAG0 as compared to controls. This was achieved most efficiently by the **DOTAP**-pYED11 complex. Thus, pYED11 may be considered as an alternative to pAG0 as a gene transfer vector.

18/3,AB/34 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1996-1999/May W1
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10325995 BIOSIS NO.: 199698780913
Structural and functional analysis of cationic transfection lipids: The hydrophobic domain.

AUTHOR: Balasubramaniam R P; Bennett M J; Aberle A M; Malone J G; Nantz M H (a); Malone R W(a)
AUTHOR ADDRESS: (a)UCD Gene Therapy Program, Departments Chemistry Medical Pathology, University California Davis, , USA

JOURNAL: Gene Therapy 3 (2):p163-172 1996

ISSN: 0969-7128

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Cationic lipids (cytoselectins) have gained widespread acceptance as pharmaceutical polynucleotide **delivery** agents for both cultured cell and in vivo transfection, and the cytoselectins **DOTAP** and DC-Cholesterol are being tested in clinical human gene therapy trials. This study reports the effects of modifications in the hydrophobic domain of a prototypic cytoselectin (DORI), including modifications in lipid side-chain length, saturation, and symmetry. A panel of related compounds

was prepared and analyzed using DNA transfection, electron microscopy, and differential scanning calorimetry (DSC). Lipid formulations were prepared with dioleoylphosphatidylethanolamine (DOPE) as unsonicated preparations and sonicated preparations. Transfection analyses were performed using cultured fibroblasts, human bronchial epithelial, and Chinese hamster ovarian cells as well as a mouse model for pulmonary gene delivery. In general, cytofectins containing dissymmetric hydrophobic domains were found to work as well or better than the best symmetric analogs. Optimal side-chain length and symmetry varied with cell type. Compounds with phase transitions (T-c) above and below physiological temperature (37 degree C) were tested for DNA transfection activity. In contrast to previous reports, cytofectin T-c was not found to be predictive of transfection efficacy. Pulmonary treatment with free DNA was found to be at least as effective as treatment with commonly used cytofectin: DNA complexes. However, cytofectins that incorporate a hydroxyethylammonium moiety in the polar domain were found to enhance in vivo gene delivery relative to free DNA.

18/3,AB/35 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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10177365 BIOSIS NO.: 199698632283
Liposome-mediated gene transfer and expression via the skin.

AUTHOR: Alexander M Yvonne; Akhurst Rosemary J(a)
AUTHOR ADDRESS: (a)Dep. Med. Genetics, Glasgow Univ., Yorkhill Hosp.,
Glasgow G3 8SJ, UK

JOURNAL: Human Molecular Genetics 4 (12):p2279-2285 1995

ISSN: 0964-6906

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A beta-galactosidase gene expression construct was used to investigate the effectiveness of gene **delivery** and expression when DNA/**liposome** complexes were topically applied to mouse skin in vivo. DNA was complexed with a commercial preparation of N-(1(2,3-dioleyloxy) propyl)-N,N,N-trimethyl-ammonium-methyl-sulphate (DOTAP) in a ratio of 1:1.6 (w/w). The DNA rapidly penetrated the skin and was expressed in the epidermis, dermis and hair follicles. A DNA concentration of 267 mu-g/ml DNA was found to be optimal for efficient transfection. Expression was seen as early as 6 h post-application, persisted at high levels 24 and 48 h post-treatment, but was markedly reduced by 7 days after application. In conclusion, utilising a commercially available **liposome** preparation, topically-applied DNA/**liposome** complexes can be efficiently **delivered** and expressed in several cell types within the skin. This simple, non-invasive technique may have implications for a number of gene therapy applications.

18/3,AB/36 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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10138024 BIOSIS NO.: 199698592942
Evaluation in vitro and in vivo of cationic **liposome**-expression construct complexes for cystic fibrosis gene therapy.

AUTHOR: McLachlan G(a); Davidson D J; Stevenson B J; Dickinson P;
Davidson-Smith H; Dorin J R; Porteous D J

AUTHOR ADDRESS: (a)Molecular Genetics Sect., MRC Human Genetics Unit,

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JOURNAL: Gene Therapy 2 (9):p614-622 1995

ISSN: 0969-7128

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We have tested the cationic **liposome**

N-(1-(2,3-dioleoyl-oxy)propyl)-N,N,N-trimethyl-ammoniummethylsulphate, (DOTAP), for gene **delivery** in vitro and in vivo with a view to clinical use in gene therapy for cystic fibrosis. **Delivery** of lacZ cDNA-DOTAP complexes via aerosol showed promoter-dependent differences in the pattern and longevity of expression. Repeated administration was well tolerated. The potential for the transfer of foreign genes into reproductive tissue was investigated by intravenous injection of DNA-DOTAP into female mice. Foreign DNA was undetectable in the ovaries by Southern blot analysis at 1 and 7 days after injection. Our results suggest that DOTAP merits testing in cystic fibrosis patients for **delivery** of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to the respiratory tract and that substitution of the cytomegalovirus (CMV) promoter for the simian virus (SV) promoter may improve on the transitory response reported previously.

18/3,AB/37 (Item 20 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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10052345 BIOSIS NO.: 199598507263

Intratracheal gene **delivery** to the mouse airway: Characterization of plasmid DNA expression and pharmacokinetics.

AUTHOR: Meyer K B; Thompson M M; Levy M Y; Barron L G; Szoka F C Jr(a)

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JOURNAL: Gene Therapy 2 (7):p450-460 1995

ISSN: 0969-7128

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Intratracheal administration of plasmid DNA resulted in gene expression in mouse airways in the absence of any enhancing agent.

Administration of plasmid DNA encoding the chloramphenicol acetyltransferase gene (CAT) in sterile water lead to CAT transgene expression that peaked between 1 and 3 days and was detected up to 28 days after DNA administration. Transgene expression was independent of mouse gender, age and strain. Levels of expression from DNA in various isotonic solutions did not differ from levels obtained with DNA administered in water, suggesting that transfection is not dependent on damage to airway cells caused by a hypo-osmotic **delivery** vehicle.

Pharmacokinetic studies using radiolabeled plasmid DNA showed that DNA was rapidly degraded, while higher levels of radioactivity were retained for longer duration following administration of cationic **liposome**-DNA complexes in the airway. Southern blot and PCR analysis confirmed that DNA complexed with DOTMA-DOPE was retained in the airways for a longer period. However, cationic liposomes DOTMA-DOPE (1:1) of DOTAP complexed with DNA, did not enhance expression over DNA alone. These results suggest that 'naked' plasmid DNA should be included as a control in all studies on intratracheal gene **delivery** using nonviral systems.

18/3,AB/38 (Item 21 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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09870267 BIOSIS NO.: 199598325185

Fusion of cationic liposomes with mammalian cells occurs after endocytosis.

AUTHOR: Wrobel Iwona; Collins David(a)

AUTHOR ADDRESS: (a)Dep. Pharmaceutics Drug Delivery, Mail Stop 8-1-A-215,
Amgen Inc., 1840 Dehavilland Drive, Thous, USA

JOURNAL: Biochimica et Biophysica Acta 1235 (2):p296-304 1995

ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The interaction of cationic liposomes prepared using either dioleoyltrimethylammonium propane (DOTAP) or 3-beta-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (DC-CHOL) with model membranes and with cultured mammalian cells was examined using an assay developed for monitoring virus-cell fusion (Stegmann et al. (1993) Biochemistry 32, 11330-11337). Lipid mixing between cationic liposomes and liposomes composed of DOPE/dioleoylphosphatidylglycerol (DOPG) or dioleoylphosphatidylcholine (DOPC)/DOPG was insensitive to pH in the range of pH 4.5-7.0 and was not affected by sodium chloride concentration in the range of 0-150 mM. Lipid mixing was dependent on dioleoylphosphatidylethanolamine (DOPE), since cationic liposomes prepared using dioleoylphosphatidylcholine (DOPC) were incapable of lipid mixing with DOPC/DOPG liposomes. The interaction of cationic liposomes with Hep G-2 and CHO D- cells was also studied. For both cell types, **liposome**-cell lipid mixing was rapid at 37 degree C, beginning within minutes and continuing for up to 1 hour after uptake. The extent of lipid mixing was decreased at 15 degree C, especially at later (gt 20 min) time points. This suggests that at least part of the observed lipid mixing occurred after reaching cellular lysosomes. No lipid mixing was seen at 4 degree C. Monensin inhibited lipid mixing between cationic liposomes and the cells, despite having no effect on **liposome** uptake. Inhibition of endocytic uptake of liposomes, either by incubation in hypertonic media or by depletion of cellular ATP with sodium azide and 2-deoxyglucose abolished **liposome**-cell fusion in both cell types. These data demonstrate that binding to the cell surface is insufficient for cationic **liposome**-cell fusion and that uptake into the endocytic pathway is required for fusion to occur.

18/3,AB/39 (Item 22 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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09545919 BIOSIS NO.: 199598000837

Oligonucleotide-cationic **liposome** interactions: A physicochemical study.

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AUTHOR ADDRESS: (a)Dep. Pharm. Technol., Univ. Kuopio, P.O. Box 1627,
FIN-70211 Kuopio, Finland

JOURNAL: Biochimica et Biophysica Acta 1195 (1):p115-123 1994

ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Cationic liposomes are effective in **delivering** antisense oligonucleotides into cells in culture, but their interactions with the oligonucleotides are poorly understood. We studied the aggregation and fusion reactions during the formation of cationic lipid/oligonucleotide complexes in solution and their interactions with lipid bilayers. Phosphorothioate oligonucleotides (15-mer) were complexed with cationic liposomes composed of dimethyldioctadecylammonium bromide (DDAB) and dioleoylphosphatidylethanolamine (DOPE) at 8:15 molar ratio or of a commercial formulation **DOTAP** (*N*-(1-(2,3-dioleyloxy)propyl)-*N,N,N*-trimethylammoniummethylsulfate), at different ratios with apparent -/+ charge ratios of 0.03-5.6. Mean size of the complexes increased with -/+ ratio so that at charge ratios 0.4-2.0 the size increased by at least an order magnitude due to the oligonucleotide induced aggregation. Resonance energy transfer experiments showed that in addition to aggregation oligonucleotides induced fusion of cationic liposomes, but the fusion was rate-controlled by the initial aggregation step. Rate constants for oligonucleotide induced aggregation were dependent on lipid concentration and were in the range of (0.2-1) $\text{cntdot } 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$ and (1-10) $\text{cntdot } 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$ for DDAB/DOPE and **DOTAP**, respectively. Increase in oligonucleotide concentration induced the aggregation and fusion until at high -/+ ratios electrostatic repulsion of negative surfaces inhibited further aggregation and fusion. **DOTAP**/oligonucleotide complexes did not induce leakage of calcein from neutral EPC liposomes, but did cause leakage at -/+ charge ratios of $\text{lt } 0.7$ and $\text{gt } 2.0$ from EPC/DOPE liposomes. Also at -/+ charge ratios below 0.8 **DOTAP**/oligonucleotide complexes induced leaking from negatively charged DPPC/DPPG liposomes. These results indicate that either phosphatidylethanolamine or negative charge are required in the cell membrane for fusion of cationic **liposome**-oligonucleotide complexes. The ratio of oligonucleotide to cationic lipid is critical in determining the physicochemical properties of the mixture.

18/3, AB/40 (Item 1 from file: 351)
DIALOG(R) File 351:DERWENT WPI
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011751962
WPI Acc No: 98-168872/199815
XRAM Acc No: C98-054049
Sandwich **liposome**(s) containing DNA between two lipid bilayers - especially for gene therapy, providing efficient **delivery**, particularly to the lungs, and high level, sustained protein expression from non-integrated DNA
Patent Assignee: US SEC DEPT HEALTH (USSH)
Inventor: PAVLAKIS G N; SMYTH-TEMPLETON N
Number of Countries: 078 Number of Patents: 002
Patent Family:
Patent No Kind Date Applcat No Kind Date Main IPC Week
WO 9807408 A1 19980226 WO 97US13599 A 19970801 A61K-009/127 199815 B
AU 9740502 A 19980306 AU 9740502 A 19970801 A61K-009/127 199830

Priority Applications (No Type Date): US 9624386 A 19960819
Language, Pages: WO 9807408 (E, 60)

Abstract (Basic): WO 9807408 A
A **liposome** composition comprises **DOTAP** (1,2-bis(oleoyloxy- 3-trimethylammonio-propane) and at least 1 of cholesterol (II) and its derivatives. Also new are: (1) DNA-sandwich liposomes (DSL) comprising lipid bilayers with DNA positioned between two or more sandwich liposomes with rho (net charge) 2 and size 200-450 nm and (2) DSL containing DNA, **DOTAP** and at least 1 of (I) or its derivatives.
The liposomes may also include (a) a biologically active agent,

especially nucleic acid and (b) at least 1 targeting ligand on the outer surface. The molar ratio DOTAP:(I) is preferably 1-3:1, and (I) may be replaced by e.g. its acetate or oleate.

USE - The liposomes are used to deliver e.g. active agents, particularly DNA for use in gene therapy, but also anticancer or other therapeutic agents, proteins, vitamins and viral or bacterial materials, hormones. They may also be used to transfect cells ex vivo for subsequent transplantation and in vaccines. Typical applications are treatment of diabetes, atherosclerosis, chemotherapy-induced multiple drug resistance, or generally immunological, neurological or viral diseases such as cancer and cystic fibrosis. The liposomes are administered by injection, orally, as aerosol, typically to provide 0.1 μg-5 mg nucleic acid/kg/day.

ADVANTAGE - The new sandwich liposome protects the active ingredient (and prevents any toxic effects on non-target tissues) and ensures highly effective in vivo delivery with a wide range of tissue distribution. Particularly high level expression of proteins is achieved (up to 150 times that for known formulations) in the lung, with sustained expression from non-integrated DNA. Large nucleic acids, e.g. plasmids or viral vectors, can be accommodated. The liposomes are very stable and have homogeneous size; can form complexes over a wide range of nucleic acid:liposome mole ratios (providing high concentration of DNA); and may include non-immunogenic targeting ligands or stealth lipids for delivery to selected sites. When used in vaccines, the liposomes increase the specific antigen response but minimise extraneous responses.

Dwg. 4/8

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| Set | Items | Description |
|-----|-------|---|
| S1 | 501 | REVIEW AND LIPOSOME? |
| S2 | 0 | S1 AND PEG (2A) CERAMIDE |
| S3 | 0 | PEG (2A) CERAMIDE |
| S4 | 0 | S1 AND PEG AND CERAMIDE |
| S5 | 5 | S1 AND PEG |
| S6 | 4 | RD (unique items) |
| S7 | 0 | S1 AND CERAMIDE |
| S8 | 0 | S1 AND DODAC |
| S9 | 0 | S1 AND DOTAP |
| S10 | 0 | DODAC AND DOTAP AND LIPID AND GENE AND DELIVER? |
| S11 | 0 | DODAC AND DOTAP AND DELIVER? |
| S12 | 8 | DODAC AND DELIVER? |
| S13 | 5 | RD (unique items) |
| S14 | 93 | DOTAP AND DELIVER? |
| S15 | 91 | S14 NOT PY>1998 |
| S16 | 0 | L15 AND CHOLESTEROL |
| S17 | 0 | L15 AND PHOPHATIDYL CHOLINE |
| S18 | 40 | S15 AND LIPOSOME |

? s peg and ceramide

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| 22562 | PEG | |
| 7553 | CERAMIDE | |
| S19 | 11 | PEG AND CERAMIDE |

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09272860 97362803

Improved DNA: **liposome** complexes for increased systemic **delivery** and gene expression.

Templeton NS; Lasic DD; Frederik PM; Strey HH; Roberts DD; Pavlakis GN
NCI-FCRDC, ABL-Basic Research Program, ~~Frederick~~, MD 21702-1201, USA.
Nat Biotechnol (UNITED STATES) Jul 1997, 15 (7) p647-52, ISSN
1087-0156 Journal Code: CQ3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To increase cationic **liposome**-mediated intravenous DNA **delivery** extruded **DOTAP**:cholesterol liposomes were used to form complexes with DNA, resulting in enhanced expression of the chloramphenicol acetyltransferase gene in most tissues examined. The DNA:**liposome** ratio, and mild sonication, heating, and extrusion steps used for **liposome** preparation were crucial for improved systemic **delivery**. Size fractionation studies showed that maximal gene expression was produced by a homogeneous population of DNA:**liposome** complexes between 200 to 450 nm in size. Cryo-electron microscopy examination demonstrates that the DNA:**liposome** complexes have a novel morphology, and that the DNA is condensed on the interior of invaginated liposomes between two lipid bilayers. This structure could account for the high efficiency of gene **delivery** *in vivo* and for the broad tissue distribution of the DNA:**liposome** complexes. Ligands can be placed on the outside of this structure to provide for targeted gene **delivery**.

before 7/24/972

18/3,AB/9 (Item 9 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09240829 96392699

Synthetic cationic amphiphile for **liposome**-mediated DNA transfection with less cytotoxicity.

Kato T; Iwamoto K; Ando H; Asakawa N; Tanaka I; Kikuchi J; Murakami Y
Department of Pharmaceutical Research, Tsukuba Research Laboratories,
Eisai Co., Ltd., Ibaraki, Japan.

Biol Pharm Bull (JAPAN) Jun 1996, 19 (6) p860-3, ISSN 0918-6158
Journal Code: BPZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A cationic peptide amphiphile comprising an L-alanine residue interposed between a charged head group and a double-chain segment, N,N-dihexadecyl-N alpha-[6-(trimethylammonio)- hexanoyl]-L-alaninamide bromide (NC5Ala2C16), was synthesized and used to prepare sonicated liposomes. We examined the efficiency of this **liposome** in gene transfer according to the transient expression of chloramphenicol acetyltransferase (CAT). This cationic **liposome** reagent facilitates efficient DNA transfection in COS-7 cells. We determined the optimum conditions for NC5Ala2C16 **liposome**-mediated transfection. The optimal amounts of the amphiphile and plasmid DNA were determined to be about 100 micrograms and 10 micrograms per 35-mm dish, respectively. The activity of this **liposome** was greater than that of commercial reagents, lipofectin, and N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium methylsulfate (**DOTAP**), and it was less toxic than lipofectin and **DOTAP** in COS-7 cells.

18/3,AB/10 (Item 10 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09086939 97281426

Evidence for safety and efficacy of **DOTAP** cationic **liposome**

that is stable in the circulation, but retains its ability to fuse with membranes. This work represents the first step toward the rationale design of fusogenic cationic liposomes for the systemic delivery of gene-based drugs to target tissues, such as tumors.

20/3,AB/5 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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10278120 BIOSIS NO.: 199698733038
Poly(ethylene glycol)-lipid conjugates regulate the calcium-induced fusion of liposomes composed of phosphatidylethanolamine and phosphatidylserine.

AUTHOR: Holland John W; Hui Cathy; Cullis Pieter R; Madden Thomas D(a)
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JOURNAL: Biochemistry 35 (8):p2618-2624 1996

ISSN: 0006-2960

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The effect of poly(ethylene glycol)-lipid (**PEG-lipid**) conjugates on liposomal fusion was investigated. Incorporation of **PEG-lipids** into large unilamellar vesicles (LUVs) composed of equimolar phosphatidylethanolamine (PE) and phosphatidylserine (PS) inhibited calcium-induced fusion. The degree of inhibition increased with increasing molar ratio of the **PEG** conjugate and with increasing size of the **PEG** moiety. Inhibition appeared to result from the steric barrier on the surface of the liposomes which opposed apposition of bilayers and interbilayer contact. In the presence of a large excess of neutral acceptor liposomes, however, fusogenic activity was restored. The rate of fusion under these conditions depended on the initial molar ratio of the **PEG** conjugate in the PE:PS vesicles and the length and degree of saturation of the acyl chains which composed the lipid anchor. These results are consistent with spontaneous transfer of the **PEG**-lipid from PE:PS LUVs to the neutral lipid sink reducing the steric barrier and allowing fusion of the PE:PS LUVs. The primary determinant of the rate of fusion was the rate of transfer of the **PEG-lipid**, indicating that liposomal fusion could be programmed by incorporation of appropriate **PEG-lipid** conjugates. Interestingly, increasing the size of the **PEG** group did not appear to affect the rate of fusion. The implications of these results with respect to the design of fusogenic liposomal drug delivery systems are discussed.

20/3,AB/6 (Item 1 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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012336505

WPI Acc No: 99-142612/199912

XRAM Acc No: C99-041623

Composition for delivering catalytic nucleic acid to cells - comprises polyethylene glycol conjugate with **ceramide** and lipid, particularly to administer ribozymes for treatment of cancer and inflammation
Patent Assignee: INEX PHARM CORP (INEX-N); RIBOZYME PHARM INC (RIBO-N)
Inventor: HOPE M J; KLIMUK S; MIN J; REYNOLDS M; SCHERRER P; SEMPLE S;
ZHANG Y

Number of Countries: 082 Number of Patents: 001

Patent Family:

| Patent No | Kind | Date | Applicant | No | Kind | Date | Main IPC | Week |
|------------|------|----------|------------|----|----------|-------------|----------|------|
| WO 9904819 | A1 | 19990204 | WO 98CA712 | A | 19980723 | A61K-047/48 | 199912 B | |

Abstract (Basic): WO 9904819 A

Composition (A) for delivering a nucleic acid catalyst (I) to a biological system comprises: (a) a poly(ethylene glycol) (PEG)-ceramide conjugate (II); (b) a lipid (III); and (c) (I). Also claimed are mammalian cells containing (A).

USE - (A) are particularly used to deliver (I), specifically ribozymes, to decrease the expression of RNA associated with mammalian disease. The composition used to deliver the ribozymes can be used to treat cancer and inflammation (claimed), as well as diseases such as, tumour angiogenesis, infections, metastasis, ocular diseases.

ADVANTAGE - (A) has improved circulation properties and serum stability, so that it can deliver (I), in vivo or in vitro, in presence or absence of serum. Particularly accumulation of (I) in the target tissue is increased because the composition is resistant to opsonisation and elimination by the mononuclear phagocytic system (contrast conventional cationic liposomes) and (A) protects (I) against nucleases.

Dwg. 0/14

20/3, AB/7 (Item 2 from file: 351)
DIALOG(R) File 351:DERWENT WPI
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012150681

WPI Acc No: 98-567593/199848

Related WPI Acc No: 96-209170

XRAM Acc No: C98-170499

New lipid formulation, and liposome containing lipid - used in delivery of bioactive agents to cells, by encapsulating agent in liposome to form liposome-bioactive complex and contacting cells with complex

Patent Assignee: UNIV BRITISH COLUMBIA (UYBR-N)

Inventor: CHOI L S L; MADDEN T D; WEBB M S

Number of Countries: 001 Number of Patents: 001

Patent Family:

| Patent No | Kind | Date | Applicat No | Kind | Date | Main IPC | Week |
|------------|------|----------|-------------|------|----------|--------------|----------|
| US 5820873 | A | 19981013 | US 94316429 | A | 19940930 | A61K-009/127 | 199848 B |
| | | | US 95486214 | A | 19950607 | | |

Priority Applications (No Type Date): US 95486214 A 19950607; US 94316429 A 19940930

Language, Pages: US 5820873 (21)

Abstract (Basic): US 5820873 A

A lipid compound of formula (I) is new. R1-R3 = H, 1-6C alkyl, acyl or aryl; R4 = H, 1-30C alkyl, 2-30C alkenyl, 2-30C alkynyl or aryl; R5 = H, alkyl, acyl, aryl or PEG; X1 = O, S or NR6, or when R5 is PEG and b is 1, then X1 is also Y1-alk-Y2; R6 = H, 1-6C alkyl, acyl or aryl; alk = 1-6C alkylene; Y = N(R7), O, S or Y1-alk-Y2; R7 = H, 1-6C alkyl, acyl or aryl; Y1, Y2 = amino, amido, carboxyl, carbamate, carbonyl, carbonate, urea or phosphoro; PEG = polyethylene glycol with an average molecular weight (Mw) of 550-8500 daltons, optionally substituted by 1-3C alkyl, alkoxy, acyl or aryl; a = 0-1; and b = 1, unless R5 is PEG, when b is 0 or 1.

USE - (II) is used in the delivery of bioactive agents to cells, by encapsulating the agent in the liposome to form a liposome-bioactive complex and contacting the cells with the complex. (II) is also used in the treatment of disease; in the delivery of vaccines, by forming a (II)-coated vaccine, and administering; and in the immunisation of patients by encapsulating an antigen in (II), and administering (all claimed).

ADVANTAGE - The PEG-modified ceramide lipids (I) enhance the properties of the liposomes (II) by: increasing the circulation longevity or lifetime of (II); preventing aggregation of the liposomes during covalent protein coupling, such as for targeting; preventing aggregation of (II) incorporating targeting groups or drugs, such as antibodies and DNA; promoting drug retention within the liposome; and increasing bilayer or other stability of (II) when low pH is required for encapsulation of bioactive agents. (I) also reduce leakage due to hydrolysis of the fatty acid chains of the liposome bilayer and are more stable than prior art lipid forms.

Dwg.4/8

20/3,AB/8 (Item 3 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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010712215

WPI Acc No: 96-209170/199621

Related WPI Acc No: 98-567593

XRAM Acc No: C96-066669

Polyethylene glycol modified ceramide lipid and liposome cpds. for delivery of bioactive agents - e.g. antitumour agents, antibiotics, immuno-modulators, anti-inflammatory agents and CNS agents

Patent Assignee: UNIV BRITISH COLUMBIA (UYBR-N)

Inventor: CHOI L S; MADDEN T D; WEBB M S; CHOI L S L

Number of Countries: 065 Number of Patents: 005

Patent Family:

| Patent No | Kind | Date | Applicat No | Kind | Date | Main IPC | Week |
|-------------|------|----------|-------------|------|----------|--------------|----------|
| WO 9610391 | A1 | 19960411 | WO 95CA556 | A | 19951002 | A61K-009/127 | 199621 B |
| AU 9535598 | A | 19960426 | AU 9535598 | A | 19951002 | | 199631 |
| EP 783297 | A1 | 19970716 | EP 95932594 | A | 19951002 | | 199733 |
| | | | WO 95CA556 | A | 19951002 | | |
| JP 10506622 | W | 19980630 | WO 95CA556 | A | 19951002 | C07C-233/18 | 199836 |
| | | | JP 96511229 | A | 19951002 | | |
| EP 783297 | B1 | 19990324 | EP 95932594 | A | 19951002 | A61K-009/127 | 199916 |
| | | | WO 95CA556 | A | 19951002 | | |

Priority Applications (No Type Date): US 94316429 A 19940930

Language, Pages: WO 9610391 (E, 61); EP 783297 (E); JP 10506622 (62); EP 783297 (E)

Abstract (Basic): WO 9610391 A

Polyethylene glycol modified ceramide lipids of formula (I) are new: R1-R3 = H, 1-6C alkyl, acyl or aryl; R4 = H, 1-30C alkyl, 2-30C alkenyl, 2-30C alkynyl or aryl; R5 = H, alkyl, acyl, aryl or PEG; R6, R7 = H, 1-6C alkyl, acyl or aryl; X1 = O, S or NR6; or when R5 = PEG and b = 1 then X1 may also be Y1-alk-Y2; Y = NR7, O, S or Y1-alk-Y2; Y1, Y2 = amino, amido, carboxyl, carbamate, carbonyl, carbonate, urea or phosphoro; alk = 1-6C alkylene; PEG = polyethylene glycol with molecular wt. 550-8500 (opt. subst. by 1-3C alkyl, alkoxy, acyl or aryl); a = 0 or 1; b = 1 unless R5 = PEG when b = 0 or 1. Also claimed is a kit for preparing labelled liposomes comprising a container with at least 2 compartments, the first contg. materials for prep. a label and the second contg. (I).

USE - The lipid is useful for forming liposomes for the delivery of bioactive agents (e.g. antitumour agents, antibiotics, immunomodulators, anti-inflammatory agents, CNS agents, oligonucleotides and vaccines, esp. vincristine).

ADVANTAGE - The novel lipids enhance the circulation longevity of the liposomes, prevent aggregation during covalent protein coupling, prevent aggregation of liposomes incorporating targeting moieties, promote drug retention within the liposome and increase bilayer stability when low pH is required for encapsulation of bioactive agents.

Dwg.0/9

20/3,AB/9 (Item 4 from file: 351)
DIALOG(R) File 351:DERWENT WPI
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008407680
WPI Acc No: 90-294681/199039
XRAM Acc No: C90-127136
XRPX Acc No: N90-226553

Monoclonal antibody against ganglioside - LAN recognise
N-acetylneuraminyl-neolactotetraosyl **ceramide**
Patent Assignee: MEIJI SEIKA KAISHA (MEIJ)
Number of Countries: 001 Number of Patents: 001
Patent Family:
Patent No Kind Date Applicat No Kind Date Main IPC Week
JP 2207798 A 19900817 JP 8927507 A 19890208 199039 B

Priority Applications (No Type Date): JP 8927507 A 19890208

Abstract (Basic): JP 2207798 A

(1) Monoclonal antibody is claimed which can recognise both
N-acetyl-neuraminyl-neolactotetraosyl **ceramide** and
N-acetylneuraminyl- lactotetraosyl **ceramide** specifically. (2)
Hybridoma which can produce monoclonal antibody of (1).
USE/ADVANTAGE - Monoclonal antibody is extremely useful for
ultramicroanalysis of NeuAc(alpha)2-3, Gal beta1-4(3)GlcNAc having
neolactotetraosyl series ganglioside and lactotetraosyl series
ganglioside antigen.

In an example, liposome was prep.. Formula used per one mouse.
alpha2-3-diaryl-p-globoside 21.6 mmol, dipalmitoylphosphatidyl choline
0.5 micro-mol, cholesterol 0.5 micro-mol, Salmonella minesota R 595 LPS
10.0 micro-g. These components were mixed, evapd. to dryness at 37
deg.C, then homogenised with CHCl3, and dried by evaporation and
further by using vacuum pump. To this, PBS (0.5 ml) was added, warmed
at 60 deg.C for 5-6 mins., then homogenised by portex mixer to prepare
liposome, the liposome was administered to 22 weeks old BALB/c mouse
(i.p.). At 3rd day after first immunisation, spleen was fused with
myeloma cell P3-X63-Ag8-U1 by 30% **PEG** 1000. After culture on HAT
selective medium, produced hybridoma was selected. By ELISA method and
LIL A method, further cloned by limiting dilution analysis. In the
result, objective hybridoma CCL3, FERM P-10468 was established. (6pp
Dwg.No.0/0)

? s reverse phase evaporation and liposome

2 REVERSE PHASE EVAPORATION
15934 LIPOSOME
S21 0 REVERSE PHASE EVAPORATION AND LIPOSOME
? s reverse phase evaporation

S22 2 REVERSE PHASE EVAPORATION
? rd

>>>Duplicate detection is not supported for File 351.

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S23 2 RD (unique items)
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23/3,AB/1 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11580176 BIOSIS NO. 199800360872

Ca²⁺ transport by reconstituted synaptosomal ATPase is associated with H⁺ countertransport and net charge displacement.

AUTHOR: Salvador Jesus M; Inesi Giuseppe; Rigaud Jean-Louis; Mata Ana M(a)
AUTHOR ADDRESS: (a)Departamento de Bioquimica y Biologia Molecular y
Genetica, Facultad de Ciencias, Universidad de, Spain

JOURNAL: Journal of Biological Chemistry 273 (29):p18230-18234 July 17,
1998

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The synaptosomal plasma membrane Ca²⁺-ATPase (PMCA) purified from pig brain was reconstituted with liposomes prepared by reverse phase evaporation at a lipid to protein ratio of 150/1 (w/w). ATP-dependent Ca²⁺ uptake and H⁺ ejection by the reconstituted proteoliposomes were demonstrated by following light absorption and fluorescence changes undergone by arsenazo III and 8-hydroxy-1,3,6-pyrene trisulfonate, respectively. Ca²⁺ uptake was increased up to 2-3-fold by the H⁺ ionophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone, consistent with relief of an inhibitory transmembrane pH gradient (ie. luminal alkalinization) generated by H⁺ countertransport. The stoichiometric ratio of Ca²⁺/H⁺ countertransport was 1.0/0.6, and the ATP/ Ca²⁺ coupling stoichiometry was 1/1 at 25degree C. The electrogenic character of the Ca²⁺/H⁺ countertransport was demonstrated by measuring light absorption changes undergone by oxonol VI. It was shown that a 20 mV steady state potential (positive on the luminal side) was formed as a consequence of net charge transfer associated with the 1/1 Ca²⁺/H⁺ countertransport. Calmodulin stimulated ATPase activity, Ca²⁺ uptake, and H⁺ ejection, demonstrating that these parameters are linked by the same mechanism of PMCA regulation.

23/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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08862515 BIOSIS NO.: 199396014016
Targeting and delivery of bactericide to adsorbed oral bacteria by use of proteoliposomes.

AUTHOR: Jones Malcolm N(a); Francis Sheila E; Hutchinson Fiona J; Handley Pauline S; Lyle Ian G

AUTHOR ADDRESS: (a)Macromolecular Organisation Biological Membranes Group, Dep. Biochemistry Molecular Biology, Uni, UK

JOURNAL: Biochimica et Biophysica Acta 1147 (2):p251-261 1993

ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Proteoliposomes having surface-bound succinylated concanavalin A (s-conA) have been prepared from a range of phospholipid mixtures by sonication (SUV) and reverse phase evaporation (REV) covering a range of size (weight-average diameter (hivin d-w)) from approx. 35 to 310 nm and weight-average number of protein molecules per liposomes (hivin P-w) from approx. 50 to 3000. The targeting of the proteoliposomes to adsorbed biofilms of the bacteria Streptococcus sanguis and Streptococcus mutans has been assessed from the extent of inhibition of an enzyme-linked immunosorbent assay (ELISA) for bacterial cell surface antigens. The surface-bound lectin enhances targeting relative to 'naked' liposomes of

thyroxine and dexam sone treatment had more pronounced effect on fetal lung DSPC content than each thyroxine and dexamethasone treatment with the same dose. These data suggested that simultaneous thyroxine and dexamethasone administration could minimize the decreases of fetal body weight, lung weight and lung protein content induced by administration of dexamethasone in the premature rats.

? s cholesterol (p) phosphatidyl choline (p) lipid

S31 0 CHOLESTEROL (P) PHOSPHATIDYL CHOLINE (P) LIPID
? s cholesterol (p) phosphatidyl choline

S32 0 CHOLESTEROL (P) PHOSPHATIDYL CHOLINE
? s cholesterol and phosphatidyl choline

205860 CHOLESTEROL
16 PHOSPHATIDYL CHOLINE
S33 2 CHOLESTEROL AND PHOSPHATIDYL CHOLINE
? rd

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34/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11816786 BIOSIS NO.: 199900062895
Lamellar lipoproteins uniquely contribute to hyperlipidemia in mice doubly deficient in apolipoprotein E and hepatic lipase.

AUTHOR: Bergeron Nathalie; Kotite Leila; Verges Marcel; Blanche Patricia;
Hamilton Robert L; Krauss Ronald M; Bensadoun Andre; Havel Richard J(a)
AUTHOR ADDRESS: (a)Cardiovasc. Res. Inst., Univ. Calif., Box 0130, San
Francisco, CA 94143, USA

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 95 (26):p15647-15652 Dec. 22, 1998

ISSN: 0027-8424

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Remnants of triglyceride-rich lipoproteins containing apolipoprotein (apo) B-48 accumulate in apo E-deficient mice, causing pronounced hypercholesterolemia. Mice doubly deficient in apo E and hepatic lipase have more pronounced hypercholesterolemia, even though remnants do not accumulate appreciably in mice deficient in hepatic lipase alone. Here we show that the doubly deficient mice manifest a unique lamellar hyperlipoproteinemia, characterized by vesicular particles 600 ANG-1,300 ANG in diameter. As seen by negative-staining electron microscopy, these lipoproteins also contain an electron-lucent region adjacent to the vesicle wall, similar to the core of typical lipoproteins. Correlative chemical analysis indicates that the vesicle wall is composed of a 1:1 molar mixture of **cholesterol** and phospholipids, whereas the electron-lucent region appears to be composed of cholesteryl esters (about 12% of the particle mass). Like the spherical lipoproteins of doubly deficient mice, the vesicular particles contain apo B-48, but they are particularly rich in apo A-IV. We propose that cholesteryl esters are removed from spherical lipoproteins of these mice by scavenger receptor B1, leaving behind polar lipid-rich particles

that fuse to form vesicular lipoproteins. Hepatic lipase may prevent such vesicular lipoproteins from accumulating in apo E-deficient mice by hydrolyzing phosphatidyl choline as scavenger receptor B1 removes the cholesteryl esters and by gradual endocytosis of lipoproteins bound to hepatic lipase on the surface of hepatocytes.

34/3,AB/2 (Item 2 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11813831 BIOSIS NO.: 199900059940
Lipolytic and oxidative changes in two Spanish pork loin products:
Dry-cured loin and pickled-cured loin.

AUTHOR: Hernandez Pilar; Navarro J L(a); Toldra F
AUTHOR ADDRESS: (a) Inst. Agroquim. y Tecnol. Alimentos, Ap. 73,
Burjassot-46100 Valencia, Spain

JOURNAL: Meat Science 51 (2):p123-128 Feb., 1999

ISSN: 0309-1740

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Lipolytic and oxidative changes were studied in two typical meat products from pork loin, dry-cured loin (DCL) and pickled-cured loin (PCL). Neither product registered changes in the percentages of the main lipid fractions: non polar lipids, phospholipids and cholesterol. However, in dry cured loin an important decrease was recorded in the main phospholipid classes, phosphatidyl choline and phosphatidyl ethanolamine. Muscle lipolytic enzymes were active in both products, and were accompanied by a significant increase in free fatty acids, from 0.580% (of total lipid) in fresh loin to 5.65 in DCL and 2.95% in PCL. With respect to oxidative changes, the peroxide value decreased in both products, and the TBA number only increased in PCL.

? s egg yolk phosphatidyl choline

S35 0 EGG YOLK PHOSPHATIDYL CHOLINE
? s egg yolk and phosphatidyl choline

2615 EGG YOLK
16 PHOSPHATIDYL CHOLINE

? s review and liposome?

456111 REVIEW
42175 LIPOSOME?
S1 501 REVIEW AND LIPOSOME?
? s s1 and peg (2a) ceramide

501 S1
0 PEG (2A) CERAMIDE
S2 0 S1 AND PEG (2A) CERAMIDE
? s peg (2a) ceramide

S3 0 PEG (2A) CERAMIDE
? s s1 and peg and ceramide

501 S1
22562 PEG
7553 CERAMIDE
S4 0 S1 AND PEG AND CERAMIDE
? s s1 and peg

501 S1
22562 PEG
S5 5 S1 AND PEG
? dr

? rd

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S6 4 RD (unique items)
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6/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09538239 98210514
PEG-liposome in DDS and clinical studies]
Maruyama K
Department of Pharmaceutics, Teikyo University.
Nippon Rinsho (JAPAN) Mar 1998, 56 (3) p632-7, ISSN 0047-1852
Journal Code: KIM
Languages: JAPANESE Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL English
Abstract
The current status of newly developed polyethyleneglycol coated
liposome (**PEG-liposome**) were described in this
review. **Liposomes** have demonstrated considerable promise as a
carrier for the delivery of drugs in vivo. However, one of the drawback is
that most **liposomes** intravenously injected into animals are rapidly
removed from the blood circulation by uptake primarily in the cells of
reticuloendothelial system (RES). It has been found that **PEG-**

liposome are not readily taken up by the macrophages the RES and hence stay in the circulation for a relatively long period of time. Pharmacokinetic analysis and therapeutic studies with tumor bearing mice revealed that **PEG-liposomes** have considerable potential as drug carriers for cancer therapy. Elevated **liposome** accumulation has been found in the tumor bearing mice model system. Results from clinical studies with doxorubicin encapsulated into **PEG-liposomes** (DOXIL) in AIDS-related Kaposi's sarcoma revealed an increased therapeutic efficacy compared to free-drug. These new formulations of long-circulating **liposomes** (**PEG-liposome**) offer the development of immunoliposomes with both long survival times in circulation and target recognition being retained in vivo. Fab'-**PEG**-immunoliposome was newly designed to gain long-circulating enough to extravasate to the targeted solid tumor in vivo. An ultimate goal of Fab'-**PEG**-immunoliposome is the incorporation of a fusogenic molecules that would induce fusion of **liposome** following their binding to the target cells or their internalization by endocytosis. Such liposomal formulations should be useful for endocytotic internalization of plasmid DNA and other bioactive materials.

6/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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09428387 98100635

An importance of colloid chemical characterization of **liposomes** for DDS in a large scale production]

Hirota S

Department of Material Science and Engineering, Tokyo Denki University, Japan.

Yakugaku Zasshi (JAPAN) Dec 1997, 117 (12) p991-1005, ISSN 0031-6903
Journal Code: JON

Languages: JAPANESE Summary Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL English
Abstract

Efficacy and safety data of liposomal drugs in a laboratory environment are often not reproduced on an industrial production scale. This is largely due to the fact that the colloid-chemical properties of the **liposomes** manufactured on a small scale are not reproduced in large scale production. Though the size and the electric charge of **liposomes** are measured and are adequately specified in relation to the bio-distributions in most developments of **liposomes** (1), uniformity of lipid components, exposure of bio-chemically important functional groups on the outer surface of **liposomes** (2), fixed aqueous layer thickness (FALT), number of the lipid bilayers, etc., are dependent upon the scale of production. Nevertheless these properties are not always exactly specified. Uniformity, especially of the functional groups on the membrane surface can be assessed chemically or bio-chemically with fractionated samples, and FALT can be easily determined through electro-chemical means (3). In this review, colloid chemical characterization of **liposomes** is introduced, FALT as an example, and its importance in a quality control of a liposomal product in an industrial scale production is shown. Methoxy-polyethyleneglycol-diacylglycerol (**PEG-DAG**) with varying **PEG** chain length and acyl chains were synthesized, FALT of **liposomes** coated with **PEG-DAG** determined and tissue distribution in tumor bearing mice. The higher incorporation ratio of **PEG-DAG** into liposomal membrane was observed with **PEG-DAG** with short acyl chains (myristoyl) and a small **PEG** molecular weight (1000). The easier to incorporate, the easier to be stripped in the serum. The disposition data in the rats well reflected the colloid chemical and in vitro data of the **PEG liposomes**. Galactosyl-carbonyl-propionyl-polyethyleneglycol-diacylglycerol (Gal-**PEG**-DAG) with oxyethylene number, n = 10, 20 and 40 were synthesized. The exposure of the galactose residue beyond the fixed aqueous layer of **liposomes** coated with Gal-**PEG-DAG** was monitored by a lectin,

Ricinus communis agglutinin (RCA) induced agglutination, the half life in the blood after i.v. injection into rats, organ distribution determined and intrahepatic distribution studied. Only the **liposomes** containing the Gal-PEG10-DAG aggregated with the lectin, indicating that only with this derivative the galactose group was adequately exposed. The Gal-PEG10-DAG **liposomes** were cleared from the plasma with a half life of 0.3 h. The plasma elimination could be attributed entirely to increased uptake by the liver. The increased liver uptake was almost entirely attributed to increased uptake by the non-parenchymal cell. Incorporation of PEG -DSPE in to the Gal-PEG10-DAG **liposomes** caused 1) a three-fold increase in blood circulation time, 2) a small but significant decrease in hepatic uptake after 20 h and 3) a significant shift in intrahepatic distribution in favor of the hepatocytes, comparable to that of the control **liposomes**. In conclusion, therapeutic efficacy and safety of **liposomes** can be controlled by their colloid chemical, more exactly, surface chemical properties. By setting up reasonable quality control specification of the properties in laboratory and examining the specifications satisfied in upscaling, the efficacy and safety are reproduced in a large scale product.

6/3,AB/3 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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11435901 BIOSIS NO.: 199800217233

Physicochemical specification of drug carrying **liposomes** for the quality control in the industrial production.

AUTHOR: Hirota Sadao(a)

AUTHOR ADDRESS: (a) 6-6-18 Higashikaigan Minami, Chigasaki 253, Japan

JOURNAL: International Journal of Pharmaceutics (Amsterdam) 162 (1-2):p
185-194 March 20, 1998

ISSN: 0378-5173

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Animal data of liposomal drugs in a laboratory environment are often not reproduced on industrial scale products. One of the most important reasons is in the fact that the colloid-chemical properties of the **liposomes** manufactured on a small scale are not reproduced in a large scale production. In this **review**, colloid chemical characterization of **liposomes** is introduced, fixed aqueous layer thickness (FALT) as an example, and its importance in a quality control of a liposomal product in an industrial scale production is shown. Methoxy-polyethylenglycol-diacylglycerol (PEGDAG) with varying PEG chain length and acyl chains were synthesized, FALT of **liposomes** coated with PEG-DAG determined and tissue distribution in tumor bearing mice studied. The higher incorporation ratio of PEG-DAG into liposomal membrane was observed with PEG-DAG with short acyl chains (myristoyl) and a small PEG molecular weight (1000). The easier to be incorporated, the easier to be stripped in the serum. The disposition data in the mice well reflected the colloid chemical and in vitro data of the **PEG liposomes**.

Galactosyl-carbonyl-propionylpolyethylenglycol-diacylglycerol (Gal-PEG-DAG) with oxyethylene number, n = 10, 20 and 40 were synthesized. The exposure of galactosy residue beyond the fixed aqueous layer of **liposomes** coated with Gal-PEG-DAG was monitored by RCA lectin induced agglutination, half life in the blood and organ distribution after i.v. injection into rats determined and intrahepatic distribution studied. Only the **liposomes** containing Gal-PEG |1|0DAG aggregated with the lectin, indicating that only with this derivative the galactose group was adequately exposed. The Gal-PEG

|1|0DAG **liposomes** were cleared from plasma with a half life of 0.3 h. The plasma elimination could be attributed entirely to increased uptake by the liver. The increased liver uptake was almost entirely attributed to increased uptake by the non-parenchymal cell. Incorporation of PEG-DSPE in the Gal-PEG|1|0DAG **liposomes** caused (1) a 3-fold increase in blood circulation time, (2) a small but significant decrease in hepatic uptake after 20 h and (3) a significant shift in intrahepatic distribution in favor of the hepatocytes, comparable to that of the control **liposomes**. In conclusion, therapeutic efficacy and safety of **liposomes** can be controlled by their colloid chemical, more exactly, surface chemical properties. By setting up reasonable quality control specifications of the properties in the laboratory and examining the specifications satisfied in upscaling, the efficacy and safety are reproduced in a large scale product.

6/3,AB/4 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R) 1969-1999/May W1
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11301731 BIOSIS NO.: 199800083063
Long-circulating immunoliposome targeting in animal models.

AUTHOR: Maruyama Kazuo(a)
AUTHOR ADDRESS: (a)Faculty Pharmaceutical Sciences, Teikyo Univ., Sagamiko, Tsukui-gun, Kanagawa 199-01, Japan

JOURNAL: Journal of Liposome Research 7 (4):p363-389 Nov., 1997

ISSN: 0898-2104

DOCUMENT TYPE: Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The current status of newly developed PEG-immunoliposomes (Type C), carrying monoclonal antibodies or their fragments (Fab') at the distal ends of the PEG chains, were described in this review. In terms of target binding of Type C, two different anatomical compartments were considered. They are the mouse lung endothelial for a readily accessible site in intravascular and the mouse implanted solid tumor for a much less accessible target site located in extravascular through leaky vascular. Distearoyl phosphatidylethanolamine derivatives of PEG with carboxyl group (DSPE-PEG-COOH) and dipalmitoyl phosphatidylethanolamine derivatives of PEG with maleimidyl group (DPPE-PEG-Mal) at the PEG's terminus were newly synthesized. Small unilamellar **liposomes** (90 - 130 nm in diameter) were prepared from phosphatidylcholine and cholesterol (2:1, m/m) containing 6 mol% of DSPE-PEG-COOH or DPPE-PEG-Mal. For targeting to the vascular endothelial surface in the lung, 34A antibody, which is highly specific to mouse pulmonary endothelial cells, was conjugated to PEG-**liposome** (34A-Type C). The degree of lung binding of 34A-Type C in BALB/c mouse was significantly higher than that of 34A-Type A which is an ordinary type of immunoliposome (without PEG derivatives). For targeting to the solid tumor tissue, 21B2 antibody which is anti-human CEA and its Fab' fragment were used. The targeting ability of Fab'-Type C was examined by using CEA-positive human gastric cancer strain MKN-45 cells inoculated into BALB/c nu/nu mice. Fab'-Type C showed the low RES uptake and the long circulation time, and resulted in enhanced accumulation of the **liposomes** in the solid tumor. The small Fab'-Type C could predominantly pass through the leaky tumor endothelium by passive convective transport. These studies offer some important insights into the potential of target-specific drug delivery.

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501 S1
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13/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09840004 99129196
Transfection of cultured myoblasts in high serum concentration with
DODAC:DOPE liposomes.
Vitiello L; Bockhold K; Joshi PB; Worton RG
CRIBI, University of Padova, Italy.
Gene Ther (ENGLAND) Oct 1998, 5 (10) p1306-13, ISSN 0969-7128

Journal Code: CCE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The inhibitory effect of serum is one of the main obstacles to the in vivo use of cationic liposomes as a DNA **delivery** system. We have found that a novel liposome formulation, **DODAC:DOPE** (1:1) is totally resistant to the inhibitory effects of serum for transfection of cultured myoblasts and myotubes. Transfection with a lacZ reporter gene in the presence of 95% fetal bovine serum gave up to 25% beta-gal-positive cells in C2C12 myoblasts and about six-fold less in primary human myoblasts. The lower transgene expression in primary cells does not appear to be a result of less DNA uptake but might result from differences in intracellular trafficking of the complexes. **DODAC**-based liposomes are unique in their resistance to serum inhibition and may therefore be valuable for the systemic **delivery** of genetic information to muscle and other tissues.

13/3,AB/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09502721 98239985

Comparison between intratracheal and intravenous administration of liposome-DNA complexes for cystic fibrosis lung gene therapy.

Griesenbach U; Chonn A; Cassady R; Hannam V; Ackerley C; Post M; Tanswell AK; Olek K; O'Brodovich H; Tsui L-C

Research Institute, MRC Group in Lung Development, Hospital of Sick Children, Toronto, ON, Canada.

Gene Ther (ENGLAND) Feb 1998, 5 (2) p181-8, ISSN 0969-7128

Journal Code: CCE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Intratracheal (i.t.) and intravenous (i.v.) **delivery** of DNA-vector formulations are two strategies to obtain gene transfer to the lung, it is still uncertain, however, which of these two modes of **delivery** will be more effective in the treatment of cystic fibrosis and other lung diseases. In this study, we attempted to optimize formulations of the cationic liposome **DODAC** :DOPE (dioleoyldimethylammonium-chloride: dioleoylphosphatidylethanolamine) complexed to plasmids encoding chloramphenicol acetyltransferase for i.t. and i.v. injection into CD-2 mice and compared the two methods. Our results showed that both methods conferred reporter gene expression in the lung that was significantly higher relative to injection of plasmid DNA alone. Expression using either mode of administration was maximal 24 h after injection and declined to around 10% of day 1 levels 2 weeks after injection. For i.v. **delivery** of **DODAC**. DOPE-DNA complexes multilamellar vesicles were more effective than large unilamellar vesicles in all organs investigated. Recombinant DNA could be detected in the distal lung region following either route of administration. However, i.t. administration predominantly led to DNA deposition in epithelial cells lining the bronchioles, e.g. in clara cells, whereas i.v. administration resulted in DNA deposition in the alveolar region of the lung including type II alveolar epithelial cells.

13/3,AB/3 (Item 3 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09405746 98115051

Cationic liposome--plasmid DNA complexes used for gene transfer retain a significant trapped volume.

Wasan EK; Fairchild A; Bally MB

Department of Advanced Therapeutics, B. C. Cancer Agency, Vancouver, Canada. ewasan@bccancer.bc.ca

J Pharm Sci (UNITED STATES) Jan 1998, 87 (1) p9-14, ISSN 0022-3549

Journal Code: JO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The goal of this study is to determine whether cationic liposomes retain any trapped volume after their complexation to plasmid DNA. This serves two purposes: to further the understanding of the physical nature of liposome/plasmid DNA complexes used in gene therapy and to investigate the potential for codelivery of other encapsulated molecules with the liposome-DNA complexes. Cationic liposomes composed of N,N-dioleoyl-N,N-dimethylammonium chloride and dioleoylphosphatidylethanolamine (**DODAC** /DOPE, 50/50 mol %) encapsulating an aqueous trap marker were used to prepare liposome-DNA complexes at various charge ratios. The trapped volume before and after DNA binding was measured by two methods: dialysis and filtration. The effect of tissue culture medium on trapped volume was also investigated. A lipid-mixing assay was employed to further characterize the aggregation events that influence trap volume. The trapped volume (V_t) of neutral control liposomes was 1.1 ± 0.04 microL/mumol, which was not affected by the addition of DNA. For cationic liposomes in the absence of DNA the V_t was 1.45 ± 0.46 and 1.54 ± 0.08 microL/mumol, as measured by the filtration and dialysis methods, respectively. After addition of DNA, the residual trapped volume (RV_t) decreased to 0.43 ± 0.1 microL/mumol and 0.47 ± 0.05 microL/mumol, as determined by each method, respectively. RV_t increased as the ratio of cationic lipid to DNA (nmol of lipid/mg of DNA) was increased above 10, a ratio that corresponds to a charge ratio (positively charged lipids to negatively charged phosphate groups) of 1.62. Aggregation and lipid-mixing were greatest at charge ratios coinciding with the lowest trapped volume. In the presence of tissue culture medium, the V_t of cationic liposomes but not neutral liposomes was reduced, suggesting that the salts have a direct effect on cationic liposomes in the absence of DNA. The RV_t of both neutral and cationic liposomes in the presence of DNA, however, was not different from that of the liposomes in the absence of DNA. These results suggest that a significant trapped volume is retained by cationic liposomes after binding to plasmid DNA. This is an important finding with regard to the potential use of DNA/liposome complexes in the codelivery of other bioactive molecules at the time of cell transfection.

13/3,AB/4 (Item 4 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08693547 96216848

Cationic lipid binding to DNA: characterization of complex formation.

Wong FM; Reimer DL; Bally MB

Division of Medical Oncology, British Columbia Cancer Agency, Vancouver, Canada.

Biochemistry (UNITED STATES) May 7 1996, 35 (18) p5756-63, ISSN 0006-2960 Journal Code: A0G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We recently demonstrated that cationic lipids, added in monomer or micellar form, bind to DNA, resulting in the formation of a hydrophobic complex. This complex can serve as a well-defined intermediate in the preparation of DNA-lipid particles (DLPs) with many potential applications for **delivery** of polynucleotides in vitro and in vivo. To develop a better understanding of the factors governing complex formation, we have characterized the cationic lipid/DNA binding reaction. This was evaluated by measuring DNA and cationic lipid (**DODAC**) complex formation using the Bligh and Dyer extraction procedure. Efficient recovery of DNA (> 95%) in the organic phase was achieved when sufficient monocationic lipids interact with DNA phosphate groups. The rate of binding depends on the amount of DNA or cationic lipid present in the system. The time required to generate the hydrophobic complex was increased when < 10 micrograms of DNA or < 40 nmol of **DODAC** was present. Surprisingly, the rate of complex

comparable concentration by factors of 2-50 depending on the liposomal lipid composition of hivin P-w. The effect of the bactericide Triclosan on the thermal properties and permeability characteristics of liposomes has been studied. At and above a molar ratio of Triclosan to lipid of 0.6, Triclosan eliminates the gel to liquid-crystalline phase transition in dipalmitoylphosphatidylcholine (DPPC) containing liposomes and increases the bilayer permeability of both liposomes and proteoliposomes to D-glucose. The proteoliposomes have been used to deliver Triclosan to S. sanguis biofilms and the inhibition of growth of the bacteria after treatment with liposomally delivered Triclosan has been determined using a microtitre plate re-growth assay and compared with growth inhibition by 'free' Triclosan. It is shown that for short exposure times (1 to 2 min) proteoliposomally delivered Triclosan is a more effective growth inhibitor than free Triclosan. The results are discussed in terms of the targeting, retention and subsequent release of Triclosan into the bacterial biofilms.

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160 BLIGH
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S24 2 BLIGH AND DYER EXTRACTION

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S25 138 BLIGH AND DYER

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30/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155: MEDLINE(R)
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08693547 96216848

Cationic **lipid** binding to DNA: characterization of complex formation.

Wong FM; Reimer DL; Bally MB

Division of Medical Oncology, British Columbia Cancer Agency, Vancouver,

Canada.

Biochemistry (UNITED STATES) May 7 1996, 35 (11) p5756-63, ISSN
0006-2960 Journal Code: AOG

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We recently demonstrated that cationic lipids, added in monomer or micellar form, bind to DNA, resulting in the formation of a hydrophobic complex. This complex can serve as a well-defined intermediate in the preparation of DNA-lipid particles (DLPs) with many potential applications for delivery of polynucleotides in vitro and in vivo. To develop a better understanding of the factors governing complex formation, we have characterized the cationic lipid/DNA binding reaction. This was evaluated by measuring DNA and cationic lipid (DODAC) complex formation using the Bligh and Dyer extraction procedure.

Efficient recovery of DNA (> 95%) in the organic phase was achieved when sufficient monocationic lipids interact with DNA phosphate groups. The rate of binding depends on the amount of DNA or cationic lipid present in the system. The time required to generate the hydrophobic complex was increased when < 10 micrograms of DNA or < 40 nmol of DODAC was present. Surprisingly, the rate of complex formation was contingent on the incubation period after partitioning the DNA/lipid mixture into organic and aqueous phases. These results suggest that the cationic lipid /DNA complex forms at the aqueous/organic interface and that DNA/lipid binding is dependent on multivalent interactions at this interface. A Scatchard analysis of DNA/DODAC binding demonstrated that the binding reaction exhibits a high degree of positive cooperativity. The apparent dissociation constant (K_d), using data obtained under conditions where DODAC binding to DNA approached saturation, indicated a high-affinity reaction ($K_d > 10^{-11}$ mol L⁻¹). At this point, approximately 8400 mol of DODAC was bound per mole of DNA, which is equivalent to a charge ratio (+/-) of 0.585 for the 7.2 kb plasmid used and suggests that formation of the hydrophobic complex occurs at a stage prior to charge neutralization. The influence of other lipids on DNA/cationic lipid binding at the aqueous/organic interface was also studied. Cholesterol and DOPC had little effect on DNA/DODAC binding while the anionic lipids LPI, DOPS, and DMPG inhibited complex formation. The zwitterionic lipid DOPE, however, had a concentration-dependent effect on cationic lipid binding that was also dependent on the mixing order. We believe that this approach for evaluating lipid /DNA binding provides an effective procedure for assessing factors which control the dissociation of lipids from DNA and may be beneficial in the selection of lipids for effective use in gene transfection studies.

30/3,AB/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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08426671 96038254

Formation of novel hydrophobic complexes between cationic lipids and plasmid DNA.

Reimer DL; Zhang Y; Kong S; Wheeler JJ; Graham RW; Bally MB
Division of Medical Oncology, British Columbia Cancer Agency, Vancouver.
Biochemistry (UNITED STATES) Oct 3 1995, 34 (39) p12877-83, ISSN
0006-2960 Journal Code: AOG

Languages: ENGLISH

Document type: JOURNAL ARTICLE

An ability to generate a well defined lipid-based carrier system for the delivery of plasmid DNA in vivo requires the characterization of factors governing DNA/lipid interactions and carrier formation. We report that a hydrophobic DNA/lipid complex can be formed following addition of cationic lipids to DNA in a Bligh and Dyer monophase consisting of chloroform/methanol/water (1:2.1:1). Subsequent partitioning of the monophase into a two-phase system allows for the extraction of DNA into the organic phase. When using monovalent cationic

lipids, such as dimethyl dioctadecylammonium bromide, dioleyl dimethylammonium chloride, and 1,2-dioleyl-3-N,N,N-trimethylaminopropyl chloride, greater than 95% of the DNA present can be recovered in the organic phase when the **lipid** is added at concentrations sufficient to neutralize DNA phosphate charge. When the polyvalent cationic lipids 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate and diheptadecylamidoglycyl spermidine are used, efficient extraction of the DNA into the organic phase is also achieved when the charge ratio between **lipid** and DNA is approximately equal. Formation of the hydrophobic DNA complex can only be achieved with cationic lipids. In the absence of added cations or in the presence of excess Ca²⁺, L-lysine, or poly(L-lysine), 100% of the DNA is recovered in the aqueous fraction. The monovalent cationic **lipid/DNA** complexes can also be prepared in the presence of detergent; however, low concentrations of NaCl (< 1 mM) lead to dissociation of the complex. Importantly, these results clearly demonstrate that cationic **lipid** binding does not lead to DNA condensation. The methods described, therefore, enable **DNA/lipid** complexes to be characterized in the absence of DNA condensation. (ABSTRACT TRUNCATED AT 250 WORDS)

30/3, AB/3 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R) 1969-1999/May W1
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06819077 BIOSIS NO.: 000088128522
EFFECTS OF PRENATAL THYROID HORMONE AND DEXAMETHASONE ADMINISTRATION ON THE LUNG SURFACTANT PRODUCTION IN PREMATURE RATS

AUTHOR: SUNG I K; LEE B C

AUTHOR ADDRESS: DEP. PEDIATR., CATHOLIC UNIV. MED. COLL., SEOUL, KOREA.

JOURNAL: J CATHOL MED COLL 42 (2). 1989. 515-526.
FULL JOURNAL NAME: Journal of Catholic Medical College
CODEN: KTUNA
RECORD TYPE: Abstract
LANGUAGE: KOREAN

ABSTRACT: Recent studies have demonstrated that the fetal endocrine system plays a major role in lung maturation. The purpose of the present study was to determine the effects of prenatal thyroxine and dexamethasone administration on the fetal body weight, lung weight, lung protein content and disaturated phosphatidylcholine (DSPC), major component of surfactant, in the premature rats. Thyroxine was injected into amniotic sac of pregnant rats with a dose of 1, 5, 10 .mu.g and dexamethasone was administered subcutaneously to pregnant rats with a dose of 100, 400, 800 .mu.g/kg body weight on gestation days 18. On the other hand, thyroxine (5 .mu.g) and dexamethasone (400 .mu.g/kg body weight) were administered to pregnant rats on gestation days 18. Premature rats were delivered by Cesarean section under ketamine anesthesia on 24 hours after the drug administration, approximately on gestation days of 19. The premature rats were killed by decapitation to allow maximal blood drainage and both lung were then dissected free from the hilum and weighed to the nearest 0.1 mg as soon as possible. DSPC content of lung was determined by the Ames method (1966) after extraction by the method of Mason et al (1976) from total **lipid** solution obtained by Bligh & Dyer method (1959), and lung protein content was determined by the Bradford method. Each treated and control group consists of 20 premature rats. Dexamethasone administration (400 and 800 .mu.g/kg) resulted in significant decreases in fetal body weight, lung weight, lung protein content compared to control rats. Thyroxine administration (10 .mu.g) resulted in significant increases in fetal lung protein content compared to control rats. Thyroxine (5 and 10 .mu.g) and dexamethasone (400 and 800 .mu.g/kg) resulted in significant increases in fetal lung DSPC content compared to control rats. And simultaneous

09883107 99184736

Contact hypersensitivity: a simple model for the characterization of disease-site targeting by liposomes.

Klimuk SK; **Semple SC**; Scherrer P; Hope MJ

Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B.C., Canada. sklimuk@inexpharm.com

Biochim Biophys Acta (NETHERLANDS) Mar 4 1999, 1417 (2) p191-201, ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A murine model of delayed-type hypersensitivity (DTH) is characterized with respect to liposome accumulation at a site of inflammation. Mice were sensitized by painting the abdominal region with a solution of 2,4-dinitrofluorobenzene (DNFB) and inflammation was induced 5 days later by challenging the ear with a dilute solution of DNFB. The inflammatory response was readily monitored by measuring ear thickness (edema) and radiolabeled leukocyte infiltration. Maximum ear swelling and cellular infiltration occurred 24 h after the epicutaneous challenge with the ear returning to normal size after approximately 72 h. We demonstrate that large unilamellar vesicles (LUV) accumulate at the site of inflammation to a level more than 20-fold higher than that measured in the untreated ear. Vesicle delivery to the ear correlated with increased vascular leakage resulting from endothelium remodeling in response to DNFB challenge, and was not a consequence of increased local tissue blood volume. Extravasation occurred only during the first 24 h after ear challenge; after this time the permeability of the endothelium to vesicles returned to normal. We further showed that LUV with a diameter of 120 nm exhibit maximum levels of accumulation, that a polyethylene glycol surface coating does not increase delivery, and that the process can be inhibited by the application of topical corticosteroids at the time of induction. These data and the inflammation model are discussed with respect to developing lipid-based drug delivery vehicles designed to accumulate at inflammatory disease sites.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155: MEDLINE(R)
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08702690 96248405

Influence of dose on liposome clearance: critical role of blood proteins.

Oja CD; **Semple SC**; Chonn A; Cullis PR

University of British Columbia, Biochemistry Department, Vancouver, Canada.

Biochim Biophys Acta (NETHERLANDS) May 22 1996, 1281 (1) p31-7, ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

It is well established that the circulation half-life of liposomes increases with increasing dose. This effect is commonly attributed to "saturation" of the fixed and free macrophages of the reticuloendothelial system resulting in reduced clearance rates. However, it is also known that the clearance rate of liposomes is dependent on the amount of associated blood protein, leading to the possibility that dose-dependent increases in circulation lifetimes could be due to decreases in the amount of blood protein associated per liposome. In order to test this hypothesis, the protein binding and clearance properties of large unilamellar liposomes composed of distearoylphosphatidylcholine/cholesterol and egg

phosphatidylcholine/diacylglycerol/phosphatidic acid/cholesterol were examined in mice. Liposomes were injected over a dose range of 10 to 1000 mg lipid/kg body weight, and the circulation lifetime and liver and spleen accumulation monitored. As expected, longer circulation half-lives were observed at higher doses for both liposome compositions. However, it was also found that at higher liposome doses, significantly less protein was bound per liposome. The results indicate that there is a limited pool of blood proteins that is able to interact with liposomes of a given composition. At higher lipid doses these blood proteins are distributed over more liposomes resulting in lower protein binding values and longer circulation lifetimes.

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08674782 96180676

Influence of cholesterol on the association of plasma proteins with liposomes.

Semple SC; Chonn A; Cullis PR

Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, British Columbia, Canada.

Biochemistry (UNITED STATES) Feb 27 1996, 35 (8) p2521-5, ISSN 0006-2960 Journal Code: A0G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The *in vivo* association of blood proteins with large unilamellar liposomes composed of saturated phosphatidylcholines was analyzed to determine the effect of membrane fluidity and hydrocarbon chain length on liposome-plasma protein interactions and liposome clearance. Liposomes composed of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and diarachidoylphosphatidylcholine (DAPC) were administered via the lateral tail vein of CD-1 mice and were subsequently isolated from the blood at 2 min postinjection. The protein binding ability (PB, grams of protein bound per mole total lipid) of the liposomes was quantified and related to their circulation half-lives. Liposomes composed of long-chain saturated phospholipids that exist in the gel (frozen) state at 39 degrees C (DPPC, DSPC and DAPC) bound large quantities of blood proteins, in excess of 48 g of protein per mole total lipid, and were found to be rapidly cleared from the circulation. The incorporation of cholesterol into DSPC liposomes resulted in significantly decreased PB values and enhanced circulation lifetimes for this lipid system. This cholesterol effect plateaued at 30 mol % cholesterol, corresponding to the loss of the gel-liquid crystalline phase transition, and resulted in PB values of 23-28 grams of protein per mole of total lipid. The types of blood proteins binding to DSPC liposomes were not significantly altered by the inclusion of cholesterol. This is the first demonstration of rapid clearance of neutral large unilamellar liposomes having high levels of bound protein.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08462828 96029683

Beta 2 glycoprotein I is a major protein associated with very rapidly cleared liposomes *in vivo*, suggesting a significant role in the immune clearance of "non-self" particles.

Chonn A; **S**e~~m~~ple SC; Cullis PR

Department of Biochemistry, University of British Columbia, Vancouver, Canada.

J Biol Chem (UNITED STATES) Oct 27 1995, 270 (43) p25845-9, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

• Document type: JOURNAL ARTICLE

Liposomes recovered from the blood of liposome-treated CD1 mice were previously reported to have a complex protein profile associated with their membranes (Chonn, A., Semple, S.C., and Cullis, P.R. (1992) *J. Biol. Chem.* 267, 18759-18765). In this study, we have further characterized and identified the major proteins associated with very rapidly cleared large unilamellar vesicles. These liposomes contained phosphatidylcholine, cholesterol, and anionic phospholipids (phosphatidylserine, phosphatidic acid, or cardiolipin) that dramatically enhance the clearance rate of liposomes from the circulation. These anionic phospholipids are normally found exclusively in the interior of cells but become expressed when cells undergo apoptosis or programmed cell death, and thus, they are believed to be markers of cell senescence. Analysis of the proteins associated with these liposomes by SDS-polyacrylamide gel electrophoresis revealed that two of the major proteins associated with the liposome membranes are proteins with electrophoretic mobilities corresponding to $M(r)$ of 66,000 and 50,000-55,000. The 66-kDa protein was identified to be serum albumin by immunoblot analysis. Using various biochemical and immunological methods, we have identified the 50-55-kDa protein as the murine equivalent of human beta 2-glycoprotein I. Beta 2-glycoprotein I has a strong affinity for phosphatidylserine, phosphatidic acid, and cardiolipin inasmuch as the levels of beta 2-glycoprotein I associated with these anionic liposomes approach or even exceed those of serum albumin, which is present in serum at a concentration 200-fold greater than beta 2-glycoprotein I. Further, we demonstrate that the amount of beta 2-glycoprotein I associated with liposomes, as quantitated by an enzyme-linked immunosorbent assay, is correlated with their clearance rates; moreover, the circulation residency time of cardiolipin-containing liposomes is extended in mice pretreated with anti-beta 2-glycoprotein I antibodies. These findings strongly suggest that beta 2-glycoprotein I plays a primary role in mediating the clearance of liposomes and, by extension, senescent cells and foreign particles.

7/3,AB/5 (Item 5 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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07147008 92406793

Association of blood proteins with large unilamellar liposomes in vivo.
Relation to circulation lifetimes.

Chonn A; Semple SC; Cullis PR

Department of Biochemistry, University of British Columbia, Vancouver,
Canada.

J Biol Chem (UNITED STATES) Sep 15 1992, 267 (26) p18759-65, ISSN
0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The proteins associated with liposomes in the circulation of mice were analyzed in order to determine whether bound proteins significantly influence the fate of liposomes in vivo. Liposomes were administered intravenously via the dorsal tail vein of CD1 mice and were isolated from blood after 2 min in the absence of coagulation inhibitors using a rapid "spin column" procedure. Various negatively charged liposomes exhibiting markedly different clearance properties were studied; notably, these included liposomes containing 10 mol % ganglioside GM1 which has been previously shown to effectively limit liposomal uptake by the fixed macrophages of the reticuloendothelial system. The protein binding ability (PB; g of protein/mol of lipid) of the liposomes was quantitated and related to the circulation half-life ($\tau_{1/2}$) of the liposomes. Liposomes having similar membrane surface charge imparted by different anionic phospholipids were found to exhibit markedly different protein binding potentials. Furthermore, PB values determined from the in vivo experiments were found to be inversely related to circulation half-lives. PB values in excess of 50 g of protein/mol of lipid were observed for rapidly cleared liposomes such as those containing cardiolipin or phosphatidic acid ($\tau_{1/2}$

1/2 less than 2 min). PB values for ganglioside GM1-containing liposomes (tau 1/2 greater than 15 h) were significantly less (PB less than 15 g of total protein/mol of total lipid). PB values were also determined for liposomes recovered from in vitro incubations with isolated human serum; relative PB values obtained from these in vitro experiments were in agreement with relative PB values measured from in vivo experiments. PB values, therefore, could be a useful parameter for predicting the clearance behavior of liposomes in the circulation. Liposomes exhibiting increased PB values in vivo were shown by immunoblot analysis to bind more immune opsonins, leading to a higher probability of phagocytic uptake. Finally, based on results obtained using the in vitro system, it is suggested that the mechanism by which ganglioside GM1 prolongs the murine circulation half-life of liposomes is by reducing the total amount of blood protein bound to the liposomes in a relatively nonspecific manner.

7/3,AB/6 (Item 6 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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06853294 92089137

Separation of large unilamellar liposomes from blood components by a spin column procedure: towards identifying plasma proteins which mediate liposome clearance in vivo.

Chonn A; Semple SC; Cullis PR
Department of Biochemistry, University of British Columbia, Vancouver Canada.

Biochim Biophys Acta (NETHERLANDS) Nov 18 1991, 1070 (1) p215-22,

ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In order to facilitate the isolation of liposomes from blood components, we have developed a simple and rapid procedure combining chromatographic and centrifugal methods. This 'spin column' procedure was used to isolate liposomes from incubation mixtures with human serum or from the blood of CD1 mice after intravenous administration of liposomes. An advantage of this procedure is that processing times are fast (typically minutes) such that the isolation procedure can be done in the absence of chelators or other coagulation inhibitors which may affect protein/liposome interactions. Furthermore, several samples can be analyzed together and small sample volumes can be processed. In addition, we show that this spin column procedure can be employed to isolate large unilamellar vesicles averaging 100 nm in diameter from lipoproteins and plasma proteins. The applicability of this spin column procedure in studying protein/liposome interactions is demonstrated by quantitating the amount of human complement component C3 bound per liposome using a C3 competitive ELISA assay after incubation with human serum. The proteins associated with the recovered liposomes were further analyzed by conventional SDS-polyacrylamide gel electrophoresis. We show that egg phosphatidylcholine/cholesterol (55:45, mol/mol) or egg phosphatidylcholine/cholesterol/dioleylphosphatidylserine (35:45:20, mol/mol) liposomes isolated from the circulation of CD1 mice within minutes of administration have distinct, complex profiles of associated proteins. By isolating circulating large unilamellar liposomes using the spin column method and characterizing the proteins associated with their membranes, this protein fingerprinting approach will expedite identifying protein interactions which affect liposome stability and clearance in vivo.

7/3,AB/7 (Item 1 from file: 5)

DIALOG(R) File 5:Biosis Previews(R)

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11675961 BIOSIS NO.: 199800457692

Stabilized antisense-lipid particles (SALP) for systemic applications:

Generation, characterization and in vivo properties.

AUTHOR: **Seemple Sean C**(a); Klimuk Sandra K; Harasym Troy O(a); Scherrer Peter(a); Santos Nancy Dos(a); Ansell Steven M(a); Lutwyche Peter(a); Hope Michael J(a)
AUTHOR ADDRESS: (a) Inex Pharmaceuticals Corp., 100-8900 Glenlyon Parkway, Glenlyon Business Park, Burnaby, BC V5J 5, Canada

JOURNAL: Journal of Liposome Research 8 (1):p104-105 Feb., 1998

CONFERENCE/MEETING: Sixth Liposome Research Days Conference Les Embiez, France May 28-31, 1998

ISSN: 0898-2104

RECORD TYPE: Citation

LANGUAGE: English

7/3,AB/8 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11630196 BIOSIS NO.: 199800411927

Interactions of liposomes and lipid-based carrier systems with blood proteins: Relation to clearance behaviour in vivo.

AUTHOR: **Seemple Sean C**(a); Chonn Arcadio; Cullis Pieter R

AUTHOR ADDRESS: (a) Inex Pharmaceuticals Corporation, 100-8900 Glenlyon Parkway, Glenlyon Business Park, Burnaby, BC, Canada

JOURNAL: Advanced Drug Delivery Reviews 32 (1-2):p3-17 June 8, 1998

ISSN: 0169-409X

DOCUMENT TYPE: Literature Review

RECORD TYPE: Citation

LANGUAGE: English

7/3,AB/9 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10294052 BIOSIS NO.: 199698748970

Liposome-blood protein interactions in relation to liposome clearance.

AUTHOR: **Seemple Sean C**; Chonn Arcadio

AUTHOR ADDRESS: INEX Pharmaceuticals Corporation, Vancouver, BC V6P 6P2, Canada

JOURNAL: Journal of Liposome Research 6 (1):p33-60 1996

ISSN: 0898-2104

DOCUMENT TYPE: Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Our recent in vivo studies have investigated the surface adsorption property of various circulating liposomes to blood proteins, and have related this property to liposome clearance behavior. In particular, we have investigated liposomes composed of different charged or neutral lipids, fatty acyl chain length and saturation, and cholesterol content. From these studies an apparent inverse relationship between the amount of blood protein that associates with large unilamellar vesicles and the circulation half-lives of the liposomes is observed, indicating that protein-mediated liposome clearance mechanisms are dominant. Furthermore, by comparing the protein profiles of rapidly cleared liposomes with liposomes exhibiting enhanced circulation times, key blood proteins have been identified and implicated in the clearance

process.

7/3,AB/10 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10108420 BIOSIS NO.: 199698563338

Beta-Glycoprotein I Is a Major Protein Associated with Very Rapidly Cleared Liposomes in Vivo, Suggesting a Significant Role in the Immune Clearance of "Non-self" Particles.

AUTHOR: Chonn Arcadio(a); **Semple Sean C**; Cullis Pieter R

AUTHOR ADDRESS: (a)INEX Pharm. Corp., 1779 West 75th Ave., Vancouver, BC V6P 6P2, Canada

JOURNAL: Journal of Biological Chemistry 270 (43):p25845-25849 1995

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Liposomes recovered from the blood of liposome-treated CD1 mice were previously reported to have a complex protein profile associated with their membranes (Chonn, A., Semple, S. C., and Cullis, P. R. (1992) J. Biol. Chem. 267, 18759-18765). In this study, we have further characterized and identified the major proteins associated with very rapidly cleared large unilamellar vesicles. These liposomes contained phosphatidylcholine, cholesterol, and anionic phospholipids (phosphatidylserine, phosphatidic acid, or cardiolipin) that dramatically enhance the clearance rate of liposomes from the circulation. These anionic phospholipids are normally found exclusively in the interior of cells but become expressed when cells undergo apoptosis or programmed cell death, and thus, they are believed to be markers of cell senescence. Analysis of the proteins associated with these liposomes by SDS-polyacrylamide gel electrophoresis revealed that two of the major proteins associated with the liposome membranes are proteins with electrophoretic mobilities corresponding to M-r of 66,000 and 50,000-55,000. The 66-kDa protein was identified to be serum albumin by immunoblot analysis. Using various biochemical and immunological methods, we have identified the 50-55-kDa protein as the murine equivalent of human beta-2-glycoprotein I. beta-2-glycoprotein I has a strong affinity for phosphatidylserine, phosphatidic acid, and cardiolipin inasmuch as the levels of beta-2-glycoprotein I associated with these anionic liposomes approach or even exceed those of serum albumin, which is present in serum at a concentration 200-fold greater than beta-2-glycoprotein I. Further, we demonstrate that the amount of beta-2-glycoprotein I associated with liposomes, as quantitated by an enzyme-linked immunosorbent assay, is correlated with their clearance rates; moreover, the circulation residency time of cardiolipin-containing liposomes is extended in mice pretreated with anti-beta-2-glycoprotein I antibodies. These findings strongly suggest that beta-2-glycoprotein I plays a primary role in mediating the clearance of liposomes and, by extension, senescent cells and foreign particles.

17/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09883107 99184736

Contact hypersensitivity: a simple model for the characterization of disease-site targeting by liposomes.

Klimuk SK; Semple SC; Scherrer P; **Hope MJ**
Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B.C., Canada. sklimuk@inexpharm.com
Biochim Biophys Acta (NETHERLANDS) Mar 4 1999, 1417 (2) p191-201,
ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH
Document type: JOURNAL ARTICLE
A murine model of delayed-type hypersensitivity (DTH) is characterized with respect to **liposome** accumulation at a site of inflammation. Mice were sensitized by painting the abdominal region with a solution of 2,4-dinitrofluorobenzene (DNFB) and inflammation was induced 5 days later by challenging the ear with a dilute solution of DNFB. The inflammatory response was readily monitored by measuring ear thickness (edema) and radiolabeled leukocyte infiltration. Maximum ear swelling and cellular infiltration occurred 24 h after the epicutaneous challenge with the ear returning to normal size after approximately 72 h. We demonstrate that large unilamellar vesicles (LUV) accumulate at the site of inflammation to a level more than 20-fold higher than that measured in the untreated ear. Vesicle delivery to the ear correlated with increased vascular leakage resulting from endothelium remodeling in response to DNFB challenge, and was not a consequence of increased local tissue blood volume. Extravasation occurred only during the first 24 h after ear challenge; after this time the permeability of the endothelium to vesicles returned to normal. We further showed that LUV with a diameter of 120 nm exhibit maximum levels of accumulation, that a polyethylene glycol surface coating does not increase delivery, and that the process can be inhibited by the application of topical corticosteroids at the time of induction. These data and the inflammation model are discussed with respect to developing lipid-based drug delivery vehicles designed to accumulate at inflammatory disease sites.

17/3,AB/2 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11675940 BIOSIS NO.: 199800457671
Systemic delivery of an encapsulated enzyme: Characterization of lipid and protein clearance in normal and immunized mice.

AUTHOR: Mok Wilson(a); Webb Murray S; **Hope Michael J**(a)
AUTHOR ADDRESS: (a)Skin Barrier Res. Lab., Div. Dermatol., Dep. Med., Univ. B.C., Vancouver, BC V6T 2B5, Canada

JOURNAL: Journal of Liposome Research 8 (1):p86 Feb., 1998

CONFERENCE/MEETING: Sixth Liposome Research Days Conference Les Embiez, France May 28-31, 1998
ISSN: 0898-2104
RECORD TYPE: Citation

LANGUAGE: English

recombinant microorganism

US PAT NO: 5,837,282 [IMAGE AVAILABLE] L20: 8 of 52
TITLE: Ionophore-mediated **liposome** loading

US PAT NO: 5,834,556 [IMAGE AVAILABLE] L20: 9 of 52
TITLE: Graft copolymer of polycationic species and water-soluble polymers, and uses therefor

US PAT NO: 5,820,873 [IMAGE AVAILABLE] L20: 10 of 52
TITLE: Polyethylene glycol modified ceramide lipids and **liposome** uses thereof

US PAT NO: 5,789,160 [IMAGE AVAILABLE] L20: 11 of 52
TITLE: Parallel selex

US PAT NO: 5,783,565 [IMAGE AVAILABLE] L20: 12 of 52
TITLE: Cationic amphiphiles containing spermine or spermidine cationic group for intracellular **delivery** of therapeutic molecules

US PAT NO: 5,773,581 [IMAGE AVAILABLE] L20: 13 of 52
TITLE: Conjugate of a solution stable G-CSF derivative and a water-soluble polymer

US PAT NO: 5,773,221 [IMAGE AVAILABLE] L20: 14 of 52
TITLE: Method of recovering a biological molecule from a recombinant microorganism

US PAT NO: 5,767,099 [IMAGE AVAILABLE] L20: 15 of 52
TITLE: Cationic amphiphiles containing amino acid or derivatized amino acid groups for intracellular **delivery** of therapeutic molecules

US PAT NO: 5,767,073 [IMAGE AVAILABLE] L20: 16 of 52
TITLE: D4 gene and methods of use thereof

US PAT NO: 5,747,471 [IMAGE AVAILABLE] L20: 17 of 52
TITLE: Cationic amphiphiles containing steroid lipophilic groups for intracellular **delivery** of therapeutic molecules

US PAT NO: 5,739,101 [IMAGE AVAILABLE] L20: 18 of 52
TITLE: Tissue factor mutants useful for the treatment of myocardial infarction and coagulopathic disorders

US PAT NO: 5,738,985 [IMAGE AVAILABLE] L20: 19 of 52
TITLE: Method for selective inactivation of viral replication

US PAT NO: 5,723,592 [IMAGE AVAILABLE] L20: 20 of 52
TITLE: Parallel selex

US PAT NO: 5,723,289 [IMAGE AVAILABLE] L20: 21 of 52
TITLE: Parallel selex

US PAT NO: 5,719,131 [IMAGE AVAILABLE] L20: 22 of 52
TITLE: Cationic amphiphiles containing dialkylamine lipophilic groups for intracellular **delivery** of therapeutic molecules

US PAT NO: 5,716,614 [IMAGE AVAILABLE] L20: 23 of 52
TITLE: Method for delivering active agents to mammalian brains in a complex with eicosapentaenoic acid or docosahexaenoic acid-conjugated polycationic carrier

US PAT NO: 5,705,385 [IMAGE AVAILABLE] L20: 24 of 52

TITLE: Lipid-nucleic acid particles prepared from a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

US PAT NO: 5,681,966 [IMAGE AVAILABLE] L20: 25 of 52
TITLE: Compounds and methods for the treatment of cardiovascular, inflammatory and immune disorders

US PAT NO: 5,674,911 [IMAGE AVAILABLE] L20: 26 of 52
TITLE: Antiinfective polyoxypropylene/polyoxyethylene copolymers and methods of use

US PAT NO: 5,653,970 [IMAGE AVAILABLE] L20: 27 of 52
TITLE: Personal product compositions comprising heteroatom containing alkyl aldonamide compounds

US PAT NO: 5,639,947 [IMAGE AVAILABLE] L20: 28 of 52
TITLE: Compositions containing glycopolypeptide multimers and methods of making same in plants

US PAT NO: 5,627,270 [IMAGE AVAILABLE] L20: 29 of 52
TITLE: Glycosylated steroid derivatives for transport across biological membranes and process for making and using same

US PAT NO: 5,622,712 [IMAGE AVAILABLE] L20: 30 of 52
TITLE: N-[.omega., (.omega.-1)-dialkyloxy]- and N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N, N, N-tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,589,363 [IMAGE AVAILABLE] L20: 31 of 52
TITLE: DNA encoding tissue factor mutants useful for the treatment of myocardial infarction and coagulopathic disorders

US PAT NO: 5,585,478 [IMAGE AVAILABLE] L20: 32 of 52
TITLE: D4 gene and methods of use thereof

US PAT NO: 5,578,442 [IMAGE AVAILABLE] L20: 33 of 52
TITLE: Graft copolymers of polycationic species and water-soluble polymers, and use therefor

US PAT NO: 5,550,289 [IMAGE AVAILABLE] L20: 34 of 52
TITLE: N-(1, (1-1)-dialkyloxy)-and N-(1, (1-1)-dialkenyloxy) alk-1-yl-N-N, N-tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,545,412 [IMAGE AVAILABLE] L20: 35 of 52
TITLE: N-[1, (1-1)-dialkyloxy]-and N-[1, (1-1)-dialkenyloxy]-alk-1-yl-n, n, n-tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,531,925 [IMAGE AVAILABLE] L20: 36 of 52
TITLE: Particles, method of preparing said particles and uses thereof

US PAT NO: 5,490,980 [IMAGE AVAILABLE] L20: 37 of 52
TITLE: Covalent bonding of active agents to skin, hair or nails

US PAT NO: 5,482,965 [IMAGE AVAILABLE] L20: 38 of 52
TITLE: Compositions and method comprising aminoalcohol derivatives as membrane penetration enhancers for physiological active agents

US PAT NO: 5,449,664 [IMAGE AVAILABLE] L20: 39 of 52

TITLE: Antivi agents

US PAT NO: 5,366,737 [IMAGE AVAILABLE] L20: 40 of 52
TITLE: N-[.omega., (.omega.-1)-dialkyloxy]- and
N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N,N,N,-
tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,346,991 [IMAGE AVAILABLE] L20: 41 of 52
TITLE: Tissue factor mutants useful for the treatment of
myocardial infarction and coagulopathic disorders

US PAT NO: 5,320,840 [IMAGE AVAILABLE] L20: 42 of 52
TITLE: Continuous release pharmaceutical compositions

US PAT NO: 5,208,036 [IMAGE AVAILABLE] L20: 43 of 52
TITLE: N-(.omega., (.omega.-1)-dialkyloxy)- and N-(.omega.,
.omega.-1)-dialkenyloxy)-alk-1-yl-N,N,N,-
tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,202,422 [IMAGE AVAILABLE] L20: 44 of 52
TITLE: Compositions containing plant-produced glycopolypeptide
multimers, multimeric proteins and method of their use

US PAT NO: 5,196,190 [IMAGE AVAILABLE] L20: 45 of 52
TITLE: Synthetic skin substitutes

US PAT NO: 5,192,749 [IMAGE AVAILABLE] L20: 46 of 52
TITLE: 4'-substituted nucleosides

US PAT NO: 5,049,389 [IMAGE AVAILABLE] L20: 47 of 52
TITLE: Novel liposome composition for the treatment of
interstitial lung diseases

US PAT NO: 5,049,386 [IMAGE AVAILABLE] L20: 48 of 52
TITLE: N-.omega., (.omega.-1)-dialkyloxy)- and
N-(.omega., (.omega.-1)-dialkenyloxy)Alk-1-YL-N,N,N,-
tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 5,043,165 [IMAGE AVAILABLE] L20: 49 of 52
TITLE: Novel liposome composition for sustained release of
steroidal drugs

US PAT NO: 4,946,787 [IMAGE AVAILABLE] L20: 50 of 52
TITLE: N-(.omega., (.omega.-1)-dialkyloxy)- and
N-(.omega., (.omega.-1)-dialkenyloxy)-alk-1-yl-N,N,N,-
tetrasubstituted ammonium **lipids** and uses therefor

US PAT NO: 4,906,476 [IMAGE AVAILABLE] L20: 51 of 52
TITLE: Novel liposome composition for sustained release of
steroidal drugs in lungs

US PAT NO: 4,897,355 [IMAGE AVAILABLE] L20: 52 of 52
TITLE: N[.omega., (.omega.-1)-dialkyloxy]- and
N-[.omega., (.omega.-1)-dialkenyloxy]-alk-1-yl-N,N,N,-
tetrasubstituted ammonium **lipids** and uses therefor

(FILE 'USPAT' ENTERED AT 18:00:35 ON 28 APR 1999)

L1 153 S PEG AND LIPID AND CATALYST AND DELIVERY
L2 5 S L1 AND PEG AND CERAMIDE
L3 18 S L1 AND PHOSPHATIDYL AND CHOLINE
L4 62 S L1 AND CHOLESTEROL
L5 14 S L3 AND CHOLESTEROL
L6 21 S L1 AND ENDONUCLEASE
L7 46 S L1 AND (RIBONUCLEOTIDE OR RNA)
L8 74 S L1 AND DNA
L9 0 S L7 AND HAMMERHEAD
L10 12 S L7 AND RIBOZYME
L11 13 S L1 AND CATIONIC LIPID
L12 0 S L1 AND DODAC
L13 0 S L1 AND DOTAP
L14 0 S (PEG (2A) CERAMIDE) (10A) CONJUGAT?
L15 4 S PEG (2A) CERAMIDE
L16 0 S L15 AND L1
L17 0 S L15 AND LIPOSOME
L18 4 S L15 AND LIPOSOME
L19 4 S L1 AND EGG YOLK

=> s 12 or 13 lr 15 or 16 or 110 or 111 or 115 or 118 or 119

MISSING OPERATOR 'L3 LR'

=> s 12 or 13 or 15 or 16 or 110 or 111 or 115 or 118 or 119

L20 52 L2 OR L3 OR L5 OR L6 OR L10 OR L11 OR L15 OR L18 OR L19

=> d ti 1-52

US PAT NO: 5,885,613 [IMAGE AVAILABLE] L20: 1 of 52
TITLE: Bilayer stabilizing components and their use in forming
programmable fusogenic **liposomes**

US PAT NO: 5,880,076 [IMAGE AVAILABLE] L20: 2 of 52
TITLE: Compositions comprising glycacarbamate and glycaurea
compounds

US PAT NO: 5,858,660 [IMAGE AVAILABLE] L20: 3 of 52
TITLE: Parallel selex

US PAT NO: 5,843,460 [IMAGE AVAILABLE] L20: 4 of 52
TITLE: Immunogenic compositions against helicobacter infection,
polypeptides for use in the compositions, and nucleic
acid sequences encoding said polypeptides

US PAT NO: 5,840,710 [IMAGE AVAILABLE] L20: 5 of 52
TITLE: Cationic amphiphiles containing ester or ether-linked
lipophilic groups for intracellular **delivery** of
therapeutic molecules

US PAT NO: 5,840,338 [IMAGE AVAILABLE] L20: 6 of 52
TITLE: Loading of biologically active solutes into polymer gels

US PAT NO: 5,837,470 [IMAGE AVAILABLE] L20: 7 of 52
TITLE: Method of recovering a biological molecule from a